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USDA FOREST SERVICE ENVIRONMENTAL ANALYSIS

FIELD TESTING OF CANDIDATE INSECTICIDES FOR CONTROL OF THE PINE BUTTERFLY

April 6, 1973

Summary Sheet

Type of Action: Administrative

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Contents

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Reviewed by Robert A. Fichter Date April 20, 1973

Recommended Approval by [signature] Date 4-20-73

Approved by Orville L. Daniels Date 4/20/73

I. DESCRIPTION

A. Proposed Action

The Pacific Southwest Forest and Range Experiment Station (USDA Forest Service) in cooperation with the Bitterroot National Forest and the Insect and Disease Branch, Division of State and Private Forestry, proposes to conduct a research field test of two insecticides against the pine butterfly, Neophasia menapia F. & F. The test area would be on the Bitterroot National Forest, Montana.

B. Test Location

The test area would be on the Bitterroot National Forest. Most of the epidemic is located along the lower face of the mountains on the west side of the Bitter Root Valley (fig. 1). The area that would be included in this test is located between Sweeney Creek (near Florence) on the north and Canyon Creek (near Hamilton) on the south. Most of the outbreak occurs in the 3,500 to 5,500 foot elevational zone. This is the area where National Forest and private land meet. Another area of heavy defoliation occurs in the Skalkaho and Sleeping Child Creek area (approximately 10 miles southeast of Hamilton). One or two study plots may be located here if sufficient plots cannot be established on the west side of the valley.

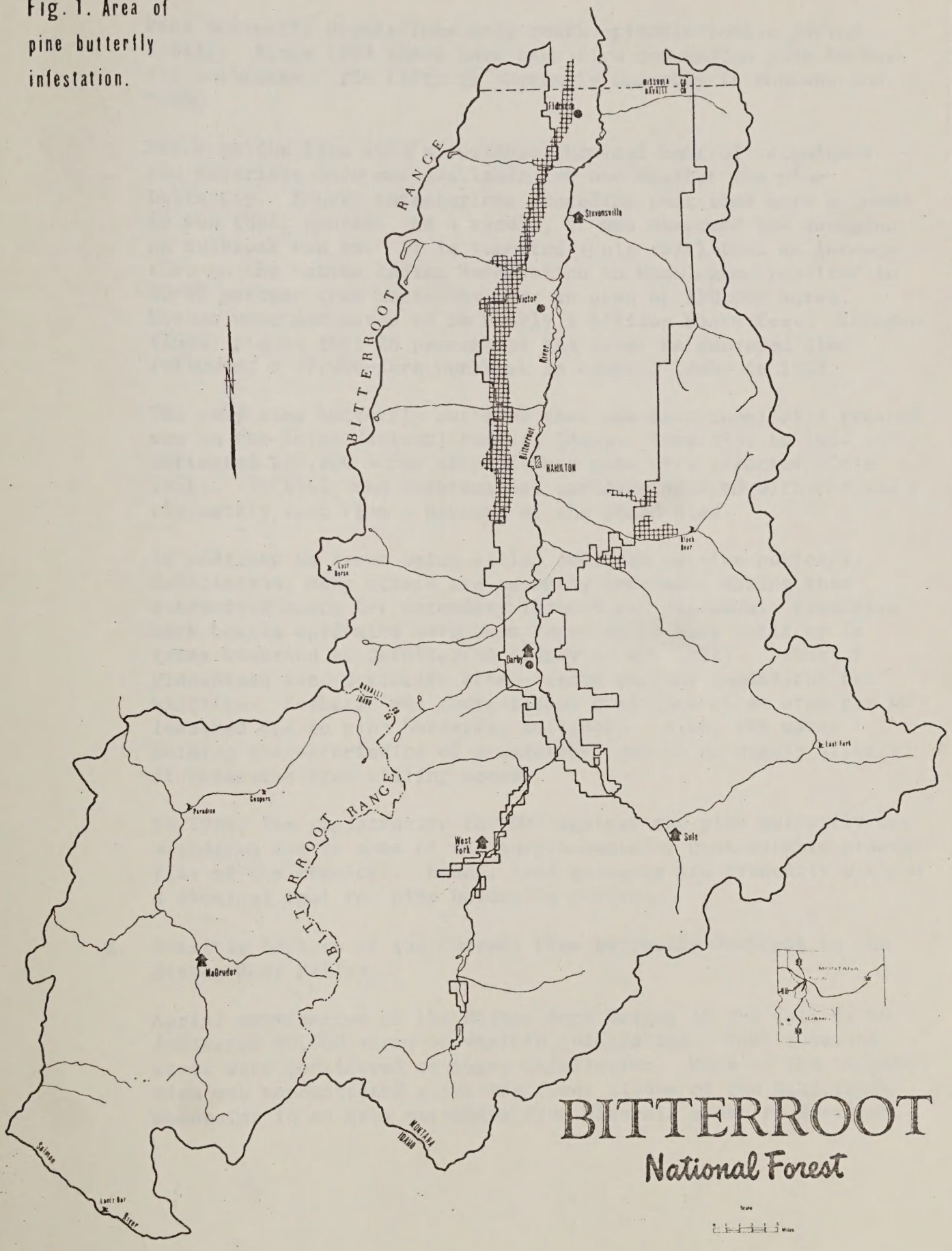
C. Time of Proposed Project

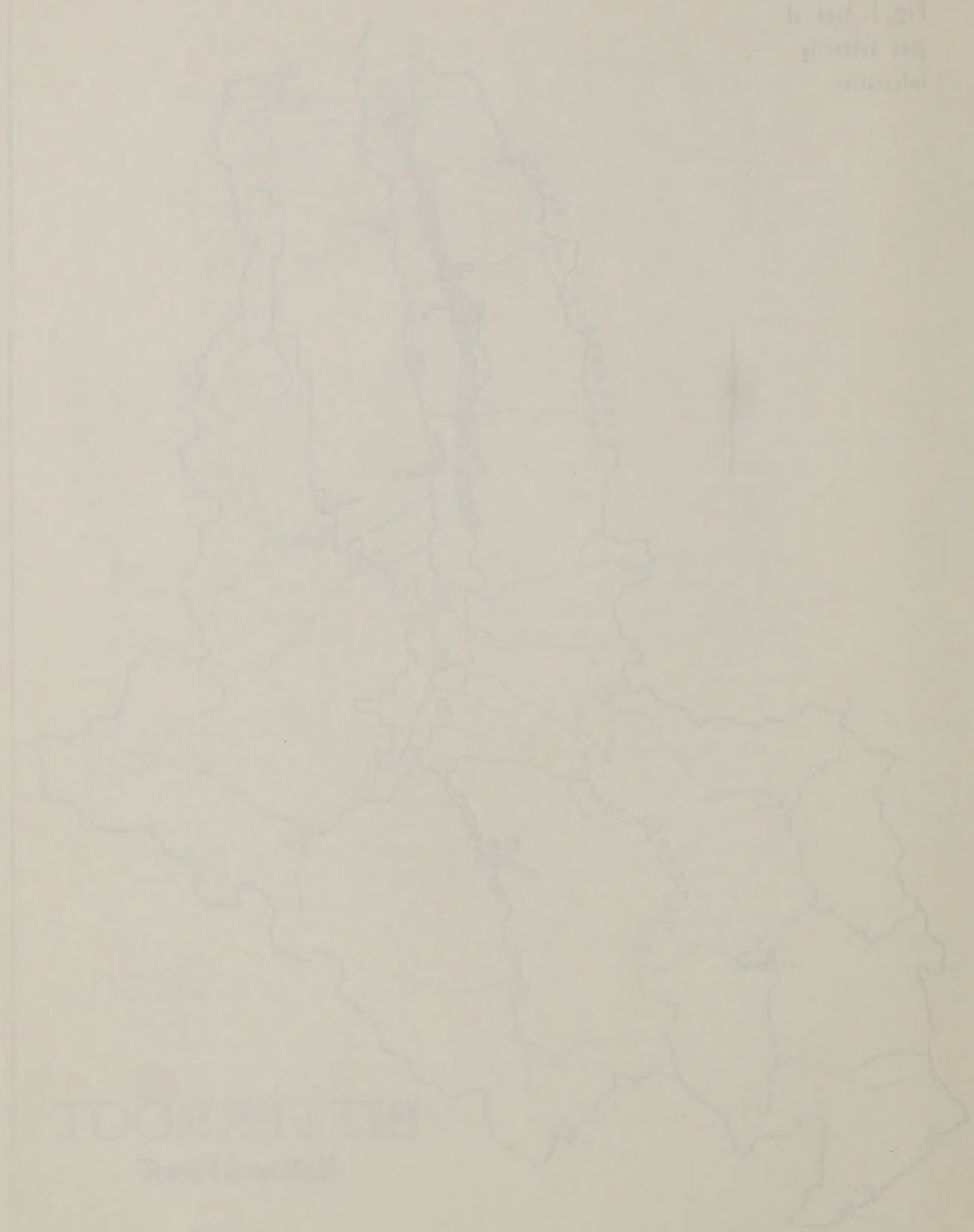
The pine butterfly is most susceptible to aerial spraying during its larval stage. The insect overwinters as an egg and hatches into the larva in late May or early June. The best time to treat it is shortly after all the eggs have hatched, while the larvae are still too small to have caused much damage. Hence, spraying is proposed for near early June for the chemical insecticides. A microbial insecticide is also being proposed for testing. Data from similar tests suggest greater success with this material when the larvae are about half grown. Thus, this material would be applied near mid-June.

D. Impact of the Pine Butterfly in Previous Outbreaks and Background

The pine butterfly is a serious defoliator of ponderosa pine, *Pinus ponderosa*, when it reaches epidemic population levels. The larvae feed on the pine needles, eventually denuding the tree resulting in significant tree mortality.

Fig. 1. Area of pine butterfly infestation.





Pine butterfly populations only reach epidemic levels periodically. Since 1893 there have been five documented pine butterfly outbreaks. The fifth is currently underway in Montana and Idaho.

Prior to the late 1940's suitable chemical control techniques and materials were not available for use against the pine butterfly. Hence, infestations preceding that time were allowed to run their course. As a result, it was observed how damaging an outbreak can be. It is reported (Cole 1971) that an infestation on the Yakima Indian Reservation in Washington resulted in 20-90 percent tree mortality over an area of 150,000 acres. Losses were estimated to be nearly 1 billion board feet. Evenden (1940) states that 26 percent of the trees he surveyed died following a 27,000-acre outbreak in central Idaho in 1922.

The only pine butterfly outbreak that has been chemically treated was on the Boise National Forest, Idaho. From 1952 to 1954 an estimated 255,400 acres of ponderosa pine were affected (Cole 1966). In 1954, the outbreak was aerially sprayed with DDT and ultimately less than 1 percent of the stand died.

In addition to trees being killed outright by pine butterfly defoliation, many others are severely weakened, making them attractive hosts for secondary insects and diseases. Extensive bark beetle epidemics have been reported to have built up in trees weakened by defoliation (Dewey et al. 1971). Areas of widespread tree mortality often create serious conditions for wildfire. Esthetic and recreational qualities of an area can be lessened due to pine butterfly outbreaks. Also, the water holding characteristics of a watershed can be adversely affected if extensive tree killing occurs.

In 1964, the registration for DDT against the pine butterfly was withdrawn due to some of the environmentally unacceptable properties of the chemical. Hence, land managers are presently without a chemical tool for pine butterfly control.

E. Possible Impacts of the Current Pine Butterfly Outbreak in the Bitter Root Valley

Aerial examination of the Bitter Root Valley in the fall of 1972 indicated 40,000 acres of visible defoliation. Four thousand acres were considered as heavy defoliation. Much of the infestation was concentrated along the lower slopes of the Bitterroot mountains in an area extending from Missoula south to Hamilton.

This area is the west side of the Bitter Root Valley. Both public and private lands are involved. Smaller, more isolated areas of infestation are also found on the east side of the valley.

Timber stand conditions vary throughout the defoliated areas. The lower elevations have ponderosa stands which are typically younger and composed of more thrifty trees. These areas were harvested in the early 1900's and regenerated naturally. Many areas not excessively overstocked are now experiencing good annual growth. Some measured stands indicate a growth rate of approximately 300 board feet per year. Potential yields in excess of 500 board feet per year could be expected if stocking control were initiated. Timber stands at the upper limits of the infestation are more typically old growth. Much of the area has been partially cut in the past. Both overstory and understory trees have been defoliated.

Often severe south exposures are heavily defoliated. These are areas in which establishment of new trees is very difficult and growth rates are slow. In a few cases the growth potential is less than 100 board feet per year.

Ornamentals are another type of affected tree. There are many rural residential dwellings along the west side of the Bitter Root Valley. Shade trees around the residences are often ponderosa pine. In areas of heavy defoliation, the trees are being as severely affected as trees under natural forest conditions.

The following summarizes the expected impact of the pine butterfly outbreak on the various resources in the Bitter Root Valley:

1. Grazing

    a. Adverse - none anticipated.

    b. Favorable - Suitable grazing areas are limited to the lower portions of the infested area. Mortality of the ponderosa pine would result in additional ground vegetation, a portion of which is expected to be suitable livestock forage. It is expected that the increase would be limited and that no substantial increase in carrying capacity would result.

2. Recreation

a. Favorable - None anticipated.

b. Adverse - Defoliation of the ponderosa pine will make many areas undesirable for recreational activities, such as camping and picnicking. Death of the trees will further degrade the areas for such uses. Dead trees in recreation areas will have to be removed for safety reasons. The numerous insects in the larval stage add to the general unattractiveness of the area.

Homeowners within the area are finding similar problems. Defoliation and mortality of shade trees will result in both an esthetic and economic loss. The presence of large numbers of insects often make the residences undesirable.

Defoliation of the ponderosa pine slopes is visible from Highway 93. It constitutes a general degradation of the visual resource.

3. Timber

a. Favorable - None.

b. Adverse - Past recorded pine butterfly infestations have caused up to 90 pereent mortality in the defoliated trees when allowed to run their course. Studies also indicate a growth loss of the surviving ponderosa pine of approximately 13 years. The reliability of any prediction of mortality and growth loss for any ongoing infestation is poor at best. It is estimated that if the infestation is allowed to run its course and does not increase in size, mortality could exceed 40,000,000 board feet and growth losses be in excess of 41,000,000 board feet.

4. Water

a. Favorable - None anticipated.

b. Adverse - None anticipated. The pine butterfly infestation is concentrated in a low water producing zone. Little impact on either quality or quantity production should result. Reduced water use because of mortality or less thrift trees should be offset by increased ground vegetation.

5. Wildlife

   a. Favorable - Limited impact.

   b. Adverse - Limited impact. Reduced tree volume will result in less escape areas but will be offset by additional forage production. Impacts on small mammals and birds is unknown.

F. Objective of Proposed Test

The objective of the proposed test is to evaluate promising non-persistent insecticides for suppression of epidemic pine butterfly populations, and to determine their effects on nontarget insects. This information is needed to lead to the registration of one or more chemicals for future pine butterfly control.

G. Selection of Candidate Insecticides

The insecticides selected for testing are Zectran and Dipel. The field testing of Dipel, however, is subject to results of some additional laboratory tests on the effects of different concentrations on the pine butterfly. If these tests indicate a specific concentration may be effective, the material will be field tested. If the laboratory test results are negative or nondefinitive, the second material to be field tested will be pyrethrin.

H. Criteria Used for Selecting Candidate Insecticides

The criteria used to select the candidate materials were:

1. Short-lived insecticide - does not persist in the environment.

2. Low toxicity to mammals, birds, and fish.

3. Laboratory tested on pine butterfly or closely related larvae and shown effective.

4. Safety tested for effects on nontarget insects.

5. Nonaccumulative - does not build up in the food chain.

6. Availability - must be available if eventually registered for use.

7. Economically feasible.

I. Background Information on the Proposed Chemicals

1. Physical and chemical properties

a. Zectran

Zectran, chemically 4-dimethyl-amino-3, 5-xylyl methyl-carbamate ($C_{12}H_{18}N_2O_2$), is a product of the Dow Chemical Company. It is a dry, white crystalline solid which melts at 85° C., is slightly water soluble (100 p.p.m.), but does not go into solution readily in most commonly used spray solvents. However, it is quite soluble in glycol ethers.

b. Dipel

Dipel is a trade name given the bacteria Bacillus thuringiensis Berliner. This is a microbial insecticide rather than a chemical. It is a rod-shaped bacteria approximately 1-micron long. It is nonsoluble in water but is suspendable. Dipel comes as a wettable powder.

c. Pyrethrin

Pyrethrin is a chemical extract of the daisy-like flower Chrysanthemum cinerariaefalium. The chemical and physical structure of the insecticide varies according to how it is manufactured (Appendix 1, pp. 636, 637).

2. Normal Residual Life

a. Zectran

Zectran is a nonpersistent insecticide, breaking down in sunlight and air within a few hours. In laboratory studies, the residual activity of Zectran and DDT was compared by spraying potted Douglas-fir trees with doses which were equivalent to 0.5 ounce/acre of Zectran and 14 ounces/acre of DDT. The deposits were allowed to age outdoors before caging insects on the treated trees. The residual life of Zectran was very short showing almost no toxicity to budworm after 2 days. The toxicity of DDT was essentially unchanged after the same length of time (Table 1) (Schmiege et al. 1970).

Table 1--Residual life of Zectran and DDT on potted Douglas-fir trees. Bioassay with 6th instar western spruce budworm[1]

Aging period outdoors	Corrected mortality (7-day post-treatment count)	
	Zectran 0.5 oz./acre	DDT 14 oz./acre
hours	--- percent of insects killed ---	
0	90	98
4	66	--
24	36	90
48	7	95

[1]Insects caged on foliage after deposit was exposed to sunlight outdoors.

Studies in the field have shown that Zectran and its breakdown products do not accumulate or persist in the environment. Residues of Zectran on Douglas-fir and five common browse plants were determined after the 1966 field tests in Montana. Zectran levels dropped rapidly in most of the plants investigated after the first day (Table 2). The amount remaining after 1 week was negligible. All Zectran residues were insignificant within a month (Pieper and Miskus 1967).

Table 2--Zectran found after period indicated (PPM by species)

ecies	Days								Weeks			Recovery factor
	0	1	2	3	4	5	6	8	2	3	4	
												percent
eudotsuga enziesii	2.86	0.19	0.22	0.15	0.14	0.17	0.14	0.17	--	0.13	--	77
lsamorhiza sp.	7.85	.47	.33	.28	.94	.82	.94	.13	0.22	.04	0.00	85
anothus sp.	1.29	1.56	.94	1.25	1.52	1.56	1.00	.67	2.01	--	.19	48
agaria sp.	6.25	4.17	4.58	2.19	5.73	2.71	3.10	--	.73	.94	.38	48
raxacum sp.	2.29	.44	.11	.11	.08	.04	.01	.13	.07	--	0.00	70
agopogon sp.	.75	.56	.29	.06	.04	--	.04	.01	.01	--	.11	80

b. Dipel

The residual life of Dipel in the environment is 7-14 days. When applied as a spray it persists in soil for less than 10 days. Its persistence in environmental waters (streams, lakes, etc.) ranges from a few hours to a few days.

c. Pyrethrin

The residual life of pyrethrin is very short--only 1 or 2 hours in sunlight. This is too short to provide satisfactory control in some cases. The pyrethrin proposed for this test has had its persistence extended to about 4 hours by stabilizing the insecticide with an antioxidant and an ultraviolet screening agent in a mineral oil formulation. This "stabilizer" acts as a sun screen.

II. ENVIRONMENTAL IMPACTS

A. Air

The previously mentioned insecticides, diluted in fuel oil or water, will be introduced into the air. To reduce drift, test guidelines prohibit their release when wind velocities exceed 5 m.p.h. Because the most sensitive areas (residences, bee yards) are to the east of the spray blocks, spraying will be terminated when winds from the west exceed 3 m.p.h. Spray will be released about 50 feet above the tree tops. The mass median diameter (m.m.d.) of the spray droplets will be 100 to 120 microns. All particles of this size should settle to the ground by gravity within 45 minutes.

Particles too small to settle will break down into nontoxic compounds within a few hours if exposed to sunlight and within about a day if not.

B. Soil

Only a fraction of the material released will ever reach the soil due to evaporation and interception by the foliage layers. Soil studies indicate that Zectran has no effect on microbial respiration when applied to soil as a dry powder, combined with soil litter, or applied in an acetone-oil carrier. Even when Zectran was applied to soil in concentrations far greater than 0.15 pound per acre, it had no significant effect on microbial activity. When properly applied, Zectran should pose no hazard to microbes in the soil (Schmiege et al. 1970). Dipel is host specific to Lepidoptera larvae (moths and butterflies) and as a result has no effect on soil micro-organisms. Pyrethrin is too unstable and short lived to have any significant impact by the time it reaches the soil when sprayed aerially.

C. Vegetation

None of the materials to be used exhibit any detectable phytotoxicity at the dosages and concentrations suggested.

Zectran levels dropped rapidly in most plants tested in a Montana study. Douglas-fir and five common browse plants were checked for residues after the 1966 field tests. The amount of Zectran remaining after 1 week was negligible except in the Fragaria and Ceanothus species (Table 3). All Zectran levels were insignificant within a month (Schmiege et al. 1970).

Table 3.--Zectran found after period indicated (p.p.m. by species)

Species	Days								Weeks			Recovery factor (percent)
	0	1	2	3	4	5	6	8	2	3	4	
Pseudotsuga menziesii	2.86	0.19	0.22	0.15	0.14	0.17	0.14	0.17	--	0.13	--	77
Balsamorhiza sp.	7.85	.47	.33	.28	.94	.82	.94	.13	.22	.04	.00	85
Ceanothus sp.	1.29	1.56	.94	1.25	1.52	1.56	1.00	.67	2.01	--	.19	48
Fragaria sp.	6.25	4.17	4.58	2.19	5.73	2.71	3.10	--	.73	.94	.38	48
Taraxaeum sp.	2.29	.44	.11	.11	.08	.04	.01	.13	.07	--	.00	70
Tragopogon sp.	.75	.56	.29	.06	.04	--	.04	.01	.01	--	.11	30

Sheridan (1972) studied the effect of Zectran on the rate of oxygen production by freshwater plants. He found "Zectran in concentrations ranging between 500 p.p.b. (parts ber billion) and approximately 5 p.p.m. (parts per million) effected total inhibition of oxygen production on $C^{14}O_2$ incorporation. The effect of Zectran on photosynthesis varied, depending on the species of alga tested." The effective life of Dipel on plants outdoors is about 7-14 days. Pyrethrin degrades within a few hours and is not retained for longer periods in vegetation.

D. Water

Although spray blocks will be selected to avoid water, there will most likely be some spray entering streams or ponds.

Water samples were collected from six small streams sprayed with Zectran on the Lolo National Forest, Montana, in 1972. A treatment of 0.15 pound Zectran in 1 gallon of fuel oil was applied per acre at a height of about 500 feet. Water samples were taken 1 hour and 24 hours after spraying. Samples were analyzed by the Environmental Protection Agency, Denver, Colorado. They detected no Zectran from the streams inside the spray area. One sample downstream from the spray area contained 0.05 parts per million Zectran. In a study on Zectran's persistence in water, Eichelberger and Lichtenberg (1971) added 10 mg. of 0.1 percent solution in acetone to a liter of water. They found that in raw river water Zectran was 85 percent degraded after 1 week and completely lost after 2 weeks. The decomposition products were also gone after 1 week.

Dipel sprayed into streams, lakes, and ponds persists from a few hours to a few days depending on temperature, pH, etc. Pyrethrin persists in water for not more than a few hours.

E. Birds and Mammals

Laboratory and field tests have been conducted by the U.S. Forest Service, the Bureau of Wildlife and Sport Fisheries (Department of Interior), Montana Fish and Game Department and others on the effects of the proposed insecticides on a variety of animals. The following are some of the findings:

1. Zectran

The acute oral, chronic feeding, and dermal toxicity of Zectran has been thoroughly investigated by Dow Chemical Co.; the U.S. Department of Interior at its Patuxent and Denver Laboratories; the U.S.D.A. Forest Service; the Department of Entomology of the University of California at Berkeley; and by Russian scientists (Schmiege et al. 1970).

The acute oral toxicity of Zectran for rats ranges from 13 to 65 milligrams per kilogram of body weight (mg./kg.). This means Zectran has a relatively high oral toxicity for mammals. Dermal toxicity, however, is extremely low. Normal handling precautions are sufficient to safeguard those working with it. Dermal toxicity was nil at 2,000 mg./kg. for rabbits.

Studies by the U.S. Fish and Wildlife Service at Denver indicate that Zectran has no cumulative effects. For example, daily doses of Zectran producing symptoms of toxicity could be tolerated by mule deer for months without any permanent detrimental effects (Tucker and Crabtree 1969).

Birds are very susceptible to the effects of many insecticides since they have very high metabolic rates. But birds have shown a high tolerance to daily doses of Zectran. While oral doses of 3.0 to 5.2 mg./kg. of Zectran killed 50 percent of a test population of mallards and chukar partridge, these birds could tolerate 40 percent of an LD/50 for 30 days. (LD/50 refers to that amount of insecticide required to kill 50 percent of the test population.) Reproduction of the treated chukars was similar to that of the controls.

Extensive studies of the effects of Zectran on wildlife were conducted by the Bureau of Sport Fisheries and Wildlife, U.S. Department of Interior, in conjunction with spruce budworm spraying. In a 3-year field study beginning in 1965, in the Bitterroot National Forest, their work showed that Zectran had no discernable effect on birds or small mammals. In general, their observations indicated that: (1) no harm to birds or mammals resulted from the Zectran applications; (2) the spray temporarily increased the availability of budworm larvae and other insect food for birds; and (3) that the reduction in available food that followed this increase

was not sufficient to cause birds to abandon their nests or to interfere with rearing of the young. The Bureau of Sport Fisheries and Wildlife concluded that over a period of 3 years, Zectran . . ."has shown no important detrimental effects to wildlife" (Schmiege et al. 1970).

The Montana Fish and Game Department investigated the effects of Zectran on grouse for a 2-year period in an area on the Bitterroot National Forest sprayed in 1966 (Mussehl and Schladweiler 1969). They also set up a special plot that was purposefully sprayed with 5 times the usual dose of 0.15 pound of Zectran per acre. With the use of banding, color marking, and radio telemetry, wildlife biologists were able to study subsequent movement of the birds. A summary of their results follows.

The strongest evidence that Zectran did not affect blue grouse survival or behavior was obtained from the multiple-sprayed Mud Creek Unit. Seven of 11 banded adult males, positively exposed to Zectran, survived to the following breeding season (10 months post-spray). An eighth banded male exposed to Zectran survived until fall (4 months post-spray), when taken by a hunter.

Radio-telemetry showed that survival and behavior of seven brood hens in the heavily sprayed area was normal for 37 days following spraying. Repeated post-spray flush counts of the broods with instrumented hens gave no indication of chick mortality in six of seven broods. Chick loss occurred in one brood shortly after spraying; whether this was a direct effect of the pesticide or natural mortality is not known.

Some observations were also made from the Trapper Creek test (1966) where Zectran was applied at 0.15 lb./acre. The area was sprayed between June 30 and July 4. Only nine of the 45 blue grouse males on Trapper Creek study units are known to have been exposed to Zectran. Five of 21 females banded on Trapper Creek units were observed after spraying. Three of the five females were radio-equipped and monitored within the sprayed area for periods of from 7 to 19 days after application. The Trapper Creek study provided the only field evaluation for the effect of Zectran on ruffed grouse. Four of five banded males were observed to have survived for varying periods after the spray. There were no significant differences in the annual survival rates of banded blue grouse males on sprayed and unsprayed study areas. In the

same period, only 15 of the more than 75 blue grouse which had been banded in the same areas were located and identified. The only ruffed grouse located in the post-spray observation period were instrumented with transmitters.

None of the instrumented grouse died or showed signs of poisoning. The movement of instrumented grouse in relation to the spray patterns was determined. Eleven of the 13 instrumented grouse exposed to Zectran remained in sprayed zones for at least a week after spraying--the period when the relatively short-lived Zectran would be potentially the most hazardous. Limited telemetry studies did not reveal changes in respiration or activity pattern of instrumented grouse after they were exposed to aerially-sprayed Zectran.

2. Dipel

Dipel is selective only to lepidopterous larvae and has no known effects on birds or animals.

3. Pyrethrin

Pyrethrin is considered as being nontoxic to birds and animals.

F. Fish and Amphibians

1. Zectran

Zectran is especially safe to fish. It is one of the least toxic chemicals tested on game fish by the U.S. Fish and Wildlife Service at their Fish Pesticide Laboratory in Denver, Colorado. For example, the toxicity of Zectran to fish is much lower than that of DDT. Toxicity with fish is measured as lethal concentration required to produce 50 percent mortality in a test population of fish (LC/50). For Zectran, the LC/50 is 5.3 milligrams per liter of water. For DDT, it is 0.002 mg./1 (Henderson et al. 1960).

Zectran has also been tested on bullfrogs, which exhibit a high tolerance to the insecticide. LD/50 ranges from 283 to 800 mg./kg. (Tucker and Crabtree 1969).

2. Dipel

There are no known effects of Dipel on fish or amphibians.

3. Pyrethrin

The effect of spraying 0.1 pound of pyrethrin in one-half gallon of carrier per acre on caged coho fingerlings was measured in Oregon (Mounts et al. 1970). They reported no apparent effects on the fish. In another test the U.S. Fish and Wildlife Service sprayed 0.2 pound of pyrethrin per acre onto a fish-bearing stream. They report no fish were killed either directly, or by eating dead insects (Anon. 1972).

Drift samples in the Oregon test contained many tadpoles. The authors state "the reason for the presence of the tadpoles is unknown, but was apparently linked to the pyrethrins" (Mounts et al. 1970).

G. Nontarget Insects

Most insects are beneficial and should not be killed. Although few, if any, insecticides are completely specific (i.e. kill only the target insect) certain precautions such as proper timing can reduce the impact of the spray on desirable species. Short lived sprays can be applied during the time of day when most nontarget insects are inactive and less likely to be contacted. Sprays can also be applied when certain nontarget species are in a developmental stage, such as eggs, that are not affected by the insecticide. Honeybee colonies can be covered or removed from the area during spraying.

1. Zectran

Although Zectran is considered somewhat selective (more toxic to lepidopterous larvae than most other species) it can affect some nontarget species. Hymenopterous (bees, wasps, ants) have been reported as being quite sensitive to Zectran.

The effect of forest spraying of Zectran on aquatic insects has been monitored frequently. In a study in Idaho, Gibson and Chapman (1966) state, "We noted no effects on benthic aquatic insects (those on the stream bottom) numbers, but observed that more insects drifted downstream for several hours beginning 3 hours after spraying . . . Adult terrestrial insects, immature Heptageneidae and Rhyacophilidae, adult Chloroperlidae, immature and adult Phrygameidae, Lumnephilidae, and Blephariceridae increased in drift samples

after spraying. We concluded that Zectran insecticide damaged aquatic organisms very little." Graham (1967) studied the effects of Zectran spraying on Piquett Creek, Bitterroot National Forest. The only effect he found was a slight increase in the number of terrestrial and drifting aquatic insects for a few hours following spraying. He concluded that with normal operational precautions, there would be little adverse effect on aquatic insects, and any effects would be temporary. Haugen (1972) monitored the effect of Zectran spraying on four streams in the Lolo National Forest, Montana. He found "a large increase in the quantity of aquatic insect drift" in one of the streams 2 to 3 hours after spraying. The other three streams monitored did not show this same increase in drift. "Postspray monitoring indicated no measurable effect of Zectran on the aquatic insect life of streams monitored."

2. Dipel

No effect to aquatic insects has been detected from Dipel. The only terrestrial insects the material effects are some members of the order Lepidoptera, and then only during the larval (caterpillar) stage.

3. Pyrethrin

Pyrethrin is a broad spectrum insecticide that is toxic to most insect species studied. Selectivity is achieved by time of spray release, and by reducing the concentration below the level that is toxic to some species. Pyrethrin can be quite damaging to aquatic insects when sprayed at concentrations that exceed 0.1 pound per acre. Following a test on the Mt. Baker National Forest in Washington, Mounts (1970) reports: "The effect of pyrethrin spray on the aquatic insect population was extremely heavy. A comparison of standing crop of aquatic insects as revealed from the stone counts showed only 1/30 as many mayflies and caddis flies remaining after treatment."

H. Visual Appearances

If the spraying is successful there will be a change in the appearance of the sprayed areas for much of the foliage will be protected. As a result, sprayed areas are expected to appear greener than unsprayed areas.

I. Human Health

Project workers will be exposed to the chemicals used. Although the proposed materials are considered to have no or a low mammalian contact toxicity, strict safety precautions will be enforced. A safety plan will be prepared and followed for each phase of the project.

III. FAVORABLE ENVIRONMENTAL EFFECTS

It is the intent of this field test to develop a safe, effective control measure for the pine butterfly. Past untreated pine butterfly infestations have resulted in from 20 to 90 percent of the stand destroyed. An outbreak in eastern Washington is reported to have killed nearly 1 billion board feet of ponderosa pine (Cole 1971).

Many of the ponderosa pine stands of Region 1 are pure stands. To destroy 20 to 90 percent of the stems can have a very detrimental effect on:

1. local economics
2. remaining trees and regeneration
3. wildlife habitat
4. water holding capabilities of the soil
5. erosion
6. esthetics
7. recreation

If the test objective is met, favorable environmental effects should result from preventing the aforementioned losses.

IV. ADVERSE ENVIRONMENTAL EFFECTS WHICH CANNOT BE AVOIDED

Even with a well-executed aerial spray project, spray deposits on nontarget areas cannot be entirely avoided. Openings and patches of nonhost timber types smaller than ¼-acre and lying within the spray block boundaries will receive the same dosage as the target areas.

Drift outside the target area is a possibility. Based upon drift tests conducted by the U.S. Army less than 10 percent of the total mass should drift outside the test areas (Barry 1973). This is

considered almost insignificant when the original concentrations are as low as those proposed (.15 pounds/acre Zectran and .10 pounds/acre pyrethrin). Past tests with the proposed materials have caused a minor reduction of some nontarget insects; however, the overall impact is insignificant for the reduction lasts for only a few days. Aquatic insect populations may be temporarily reduced if Zectran or pyrethrin should drift into streams; however, large numbers will survive to restore the species to full biotic potential (Bollen et al. 1970). There should be no effect on the fisheries. Microarthropods and other decomposers may temporarily suffer slight reductions in numbers in areas sprayed with Zectran and pyrethrin but their recovery is rapid and should be complete in 1 or 2 weeks.

V. ALTERNATIVES TO THE PROPOSED ACTION

The following alternatives to the proposed action must be considered:

A. Allow pine butterfly infestations to run their course (do nothing).

B. Develop cultural controls

C. Develop other biological controls

D. Develop controls that influence pine butterfly behavior

1. Sex attractants

2. Growth regulators

3. Sound attractants

Alternative A.

There undoubtedly are times when this would be the most logical course of action to take.

This alternative is unacceptable for the objective of the test is not to control the current outbreak or protect the resources affected, but to collect needed data. If we do not develop a chemical control method now, we will not have to the option of control in areas where control is advisable in the future.

Alternative B.

This would have to be a very long-range goal, for cultural controls that show promise; i.e., thinning, pruning, maintaining thrifty fast-growing trees, etc., take long periods of time to implement over large acreages. In Region 1, where such a large percent of our timber is still only lightly managed, it is unrealistic to hope for cultural control of pine butterflies for years to come.

Alternative C.

The only biological control that has been developed sufficiently to field test is the one proposed, Bacillus thuringiensis. Other biological controls have promise, but require much additional research in their development. Natural enemies (parasites and predators) have been responsible for the collapse of past pine butterfly outbreaks. Unfortunately, these insects lag behind butterfly outbreaks by about 2 years. By this time, permanent injury has resulted to the trees. Present technology is not sufficiently advanced to use predators and parasites for early control of pine butterfly infestations. Much research would be needed before this tool can be available.

Alternative D.

To date no research has been conducted to manipulate pine butterfly populations influencing their mating, feedings, or flight behavior. Until technology is developed along these lines by research there can be no field testing or control work using these techniques.

VI. THE RELATIONSHIP BETWEEN LOCAL SHORT-TERM USES OF MAN'S ENVIRONMENT AND THE MAINTENANCE AND ENHANCEMENT OF LONG-TERM PRODUCTIVITY

The attitude of the Forest Service is that it is its responsibility to protect the resources on National Forest lands for future generations. These resources include:

1. clean air
2. stable soils
3. clean water
4. timber
5. food and cover for wildlife
6. food for domestic livestock
7. natural beauty

The proposed action will have almost no immediate effect on the above resources for the proposal is to conduct a small scale experiment. The total area to be sprayed, 480 acres (twelve 40-acre blocks) is too small to significantly affect the long term productivity of the resources. However, if the field test is successful and leads to the registration of one or more materials for pine butterfly control,

then circumstances may dictate control operations in future years. Any future project will have to be evaluated separately considering the material to be used and the area to be treated before an estimate of the long range effects on the environment can be determined.

VII. IRREVERSIBLE AND IRRETRIEVABLE COMMITMENTS OF THE RESOURCES

There will be no such commitments as a result of the proposed action.

VIII. CONSULTATION WITH OTHERS

A. Agencies with input into the plan

Representatives of the following agencies have had an input into this environmental analysis.

1. Insecticide Evaluation Project, Pacific Southwest Forest and Range Experiment Station
2. Intermountain Forest and Range Experiment Station
3. Pacific Northwest Forest and Range Experiment Station
4. Bitterroot National Forest
5. Division of Forestry, Department of Natural Resources and Conservation, State of Montana
6. Forest Insect and Disease Research, U.S. Forest Service, Washington, DC
7. Forest Insect and Disease Administration, U.S. Forest Service, Washington, DC
8. Division of Information and Education, U.S. Forest Service, Missoula, Montana

B. Agencies to Review the Plan

Copies of this environmental analysis are being sent for comment and review to those listed above and the following:

1. Bureau of Sport Fisheries and Wildlife, Denver, Colorado
2. Montana State Fish and Game Department, Helena, Montana
3. Montana Environmental Council, Helena, Montana

4. School of Forestry, University of Montana, Missoula, Montana

5. Western Montana Wildlife Federation, Missoula, Montana

6. Missoula Chapter Sierra Club, Missoula, Montana

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APPENDIX

APPENDIX 1

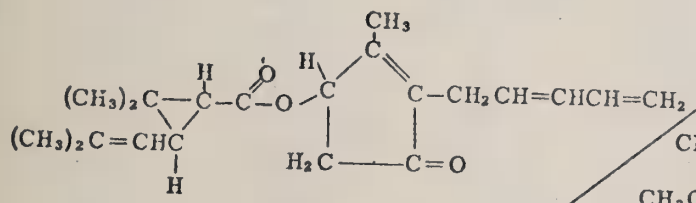
BACKGROUND INFORMATION ON PYRETHRINS

154

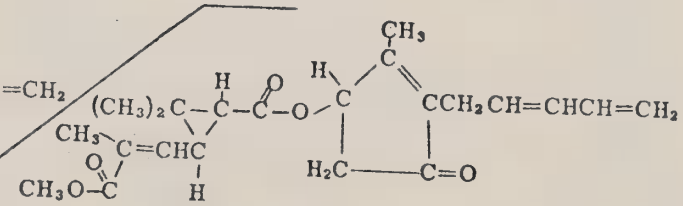
PYRETHRINS I, II; PYRETHRUM (Cinerolone / Pyrethrolone esters of mono- and di-chrysanthemum carboxylic acids; Dalmatian Insect Flowers; Powder or extracts of Chrysanthemum cinerariaefolium)

N.B.

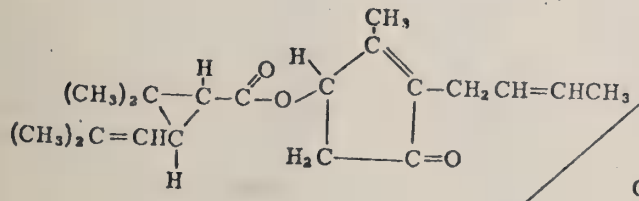
By pyrethrins I, II, is to be understood: For pyrethrin I, the esters pyrethrin I and cinerin I; for pyrethrin II: The esters pyrethrin II and cinerin II, occurring in various proportions in the natural product, pyrethrum, depending on the strain of Chrysanthemum cinerariaefolium, locale and circumstances of cultivation, techniques of extraction and condensation. 1897 2956,1893 1894,1896



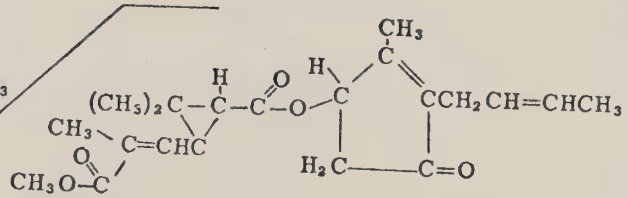
Pyrethrin I



Pyrethrin II



Cinerin I



Cinerin II

Thus, it is evident that the active principles of extracts of pyrethrum flowers are: For pyrethrin I and cinerin I, esters respectively of the alcohols pyrethrolone and cinerolone and chrysanthemum monocarboxylic acid; for pyrethrin II and cinerin II, esters respectively of pyrethrolone and cinerolone and chrysanthemum dicarboxylic acid monomethyl ester.

GENERAL (Also consult Allethrin, Cyclethrin, Furethrin, Synergism) [Refs.: 353, 2231, 2815, 757, 1059, 2226, 2120, 129, 151, 977, 851, 1801]

The insecticidal principles of pyrethrum are among the most useful and safe of all insect toxicants being noted for an extraordinary rapidity of action and a wide "spectrum" of activity to diverse insect species. The complex of substances which constitutes "pyrethrum," in the broad sense, occurs in nature in the genus Chrysanthemum

(= Pyrethrum) (Compositae), and particularly in Chrysanthemum cinerariaefolium and Chrysanthemum coccineum, plants whose insecticidal properties have long been known. Pyrethrin content ranges from 1.3 - 3% (flowers from Kenya) 1% (flowers from Japan) 0.7% (flowers from Dalmatia). The active principles reach their greatest concentration in mature flower heads (ca one-tenth as much is present in the stems) with the achenes being the principal site of concentration. The active principles have been synthesized and a series of purely synthetic analogues elucidated as well. These are potent, direct contact insecticides, producing rapid paralysis, but lacking in persistent or residual properties. The pyrethrins yield the most convincing evidences of synergism with various compounds.

PHYSICAL, CHEMICAL

[Refs.: 2956, 462, 463, 1396, 652, 1398, 250, 653, 2923, 1894, 1895, 1896, 1897, 3286, 689, 2752, 651, 1205, 1206, 251, 668, 2754, 2467, 3335]

Pyrethrins I and II are viscous, brown, liquid oleoresins; b.p. I = 170°C at 0.1 mm Hg, with decomposition; II = 200°C at 0.1 mm Hg, with decomposition; n_D: I = 1.5192 at 18°C; II = 1.529 at 21.5°C; both are virtually insoluble in water, but are soluble in many organic solvents, for instance alcohol, petroleum ether (II less than I), kerosene, carbon tetrachloride, ethylene dichloride, nitromethane; rapidly oxidized and inactivated in air; decomposed by exposure to light with loss of insecticidal activity; the constituents: Pyrethrolone = d-2-cis-(penta-2',4'-dienyl)-3-methyl-cyclopent-2-en-4-ol-1-one (d-cis-penta-2,4-dienylrethrolone); cinerolone = d-2-cis-(but-2'-enyl)-3-methyl-cyclopent-2-en-4-ol-1-one (d-cis-but-2-enylrethrolone) b.p. respectively 110°-112° at 0.1 mm Hg, 120°-124° at 1 - 2 mm Hg; pyrethrolone and cinerolone exist in optically active and racemic form; chrysanthemum monocarboxylic acid (chrysanthemic acid) = 2,2-dimethyl-3-isobutylene cyclopropene-1-carboxylic acid b.p. 135° at 12 mm Hg; chrysanthemum dicarboxylic acid monomethyl ester (pyrethric acid) b.p. 140°C at 0.5 mm Hg; the two acids may exist as stereo- and geometric-isomers, for example dl-transchrysanthemic acid (m.p. 54°C) dl-cis-chrysanthemic acid (m.p. 115°-116°C) l-trans-chrysanthemic acid (m.p. 19°C) have been synthesized in crystalline form; d-trans-chrysanthemic acid (m.p. 17°-21°C) has been identified with the acid of natural pyrethrins and d-cis chrysanthemic acid (m.p. 40°-42°C) and l-cis-chrysanthemic acid (m.p. 41°-43°) have been recovered from racemates; the naturally occurring form of pyrethric acid is also d-trans.; flowers (in the unground state) are more stable in air and light than the pulverized product; antioxidants usefully protect insecticidal residues of pyrethrins, for instance pyrocatechol, pyrogallol, hydroquinone; benzene-azo-β-naphthol exercises a protectant effect in sunlight; most of the insecticidal action is destroyed by minor changes in the pyrethrin or cinerin molecules.

a) Formulations: Dusts (ground flower heads) in non-alkaline carriers; aerosols in volatile liquids; combined with synergists in aerosols; extracts as sprays in suitable solvents.

TOXICOLOGICAL

1) Acute toxicity for higher animals:

a) Average LD_{50} oral.(acute) for all animals tested = 1500 mg/k, the chronic MLC = 500 ppm. 1949

Animal	Route	Dose	Dosage (mg/k)	Remarks	
Mouse	ip	LD_{100}	200	Given in petroleum oil.	2827
Mouse	ip	LD_{50}	40	Pyrethrin II in lauryl glycol.	1127
Mouse	ip	LD_{100}	60	" "	1127
Mouse	ip	LD_{50}(ca) = LD_{66}	240	Pyrethrin II in sesame oil; death in 21-141 min	1971
Mouse	ip	LD_{100}	>480	" "	1951
Mouse	ip	LD_{83}(ca)	480	Pyrethrins I, II in sesame oil.	1951
Rat	or	LD_{50}	820(680-1000)	In a 20% oil base.	478
Rat	or	LD_{50}	1870(1340-2600)	"	478
Rat	or	LD_{50}	ca 1500		1949
Rat	ip	LD_{80}	200	In petroleum oil.	2827
Guinea Pig	ip	LD_{80}	200	"	2827
Guinea Pig	ip	LD_{50}	100-150		1971
Guinea Pig	ip	LD_{100}	120	Pyrethrin I; in lauryl glycol.	1127
Guinea Pig	or	LD_{50}	1500	Death in 48 hrs.	1971
Dog	iv	LD	6-8		536
Fish	Medium	Toxic Dose	20 ppm (0.2 ppm pyrethrins)	As pyrethrum flowers.	174,939
Carp	Medium	Harmful	2 ppm	Affects movements. As pyrethrum flowers.	174,939
Carp	Medium	LC	5-10 ppm	Paralysis before death. "	174,939
Trout (fingerling)	Medium	Disabling Dose	5-10 ppm (0.1 ppm pyrethrins)		3175
Acellus aquaticus (Crustacea)	Medium	LC	0.002 ppm	As pyrethrum flowers.	3175

2) Sub-acute and chronic toxicity for higher animals:

a) Rats: Received 1000 ppm in diet for 2 years without tissue pathology. 54

b) Rats: Received 5000 ppm in diet: Tissue damage and gross signs in one series, but not in another. 54

c) Rabbits: Dermal applications at 200-400 mg/k gave toxic signs. 1971

d) Guinea Pigs, Rats: Survived 480 mg/animal, with diarrhoea as only symptom. 1971

e) Rabbits: Survived, with permanent tremor and spastic incoordination, 240 mg/k of pyrethrin II. 1971

f) Irritating to eyes and mucous membranes; dermatitis in some hypersensitive human subjects. Allergic reactions in sensitive subjects. 2132

3) Pharmacological, pharmacodynamical, physiological, etc.; higher animals: 851

a) Reported to be more toxic to man by inhalation than by other routes, with pyrethrin II being less toxic than pyrethrin I. 2221

(1) Sternutatory and bitter in taste when aerosols are overused. 851

b) Symptoms of acute poisoning are: Hyperexcitability, incoordination, convulsions, with death in respiratory paralysis. Oral toxicity is low for mammals and is stated to present less of a hazard than pyrethrin solvents. Chronic poisoning is said to be unlikely. 851

c) In mice, the threshold of a peculiar convulsant action, "dancing in place", is reported as 20 mg/k for pyrethrins I and II. 1971

4) Phytotoxicity:

a) Apparently non-phytotoxic; no reports of phytotoxicity with pyrethrins, as such, appear in the "literature". Solvents of pyrethrins, improperly applied, may present a hazard as is the case with many insecticide formulations.

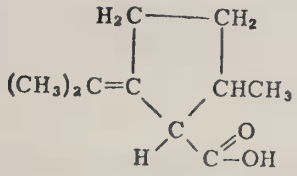
5) Toxicity for insects:

a) Pyrethrolone and cinerolone (keto-alcohol moieties) and the chrysanthemum mono- and di-carboxylic acid moieties of the pyrethrins in uncombined form are non-toxic to insects. 934,2956

(1) No esters compare with the naturally occurring esters in general insecticidal activity. 2955,3058

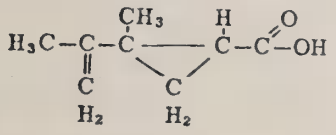
(2) Esters of acetic, isobutyric, undecylenic, malonic, benzoic, o-methoxybenzoic, cinnamic, geranium, camphor ester, β-fencholic, citronellic, piñon, crotonic, dichloroacrylic acids are all inactive. 2956,1443

(3) Esters of pulegenic acid with pyrethrolone and cinerolone are weakly active insecticides: 2956



= pulegenic acid;

and of all tested modifications of the acid moiety, esters of the following acids with pyrethrolone, cinerolone proved the most active:



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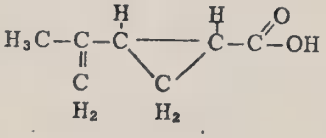


b) Structure and Insecticidal Activity: 934

(1) Relative toxicity of pyrethrum components for Musca domestica: 1147,2231

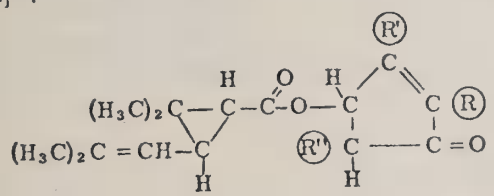
Component (As kerosene sprays)	[Ref. 1147] Relative Toxicity (Pyrethrin I = 1)	Ratio	[Ref. 2231] Relative Toxicity (Pyrethrins = 1)
Pyrethrin I	1.0	1	2.0
Pyrethrin II	4.3	0.25	0.46
Cinerin I	1.4	0.69	1.38
Cinerin II	5.8	0.17	0.35
d-cis-Cineronyl-d-trans-chrysanthemate	---	---	0.67
l-cis-Cineronyl-d-trans-chrysanthemate	---	---	1.22
d-cis-Cineronyl-l-trans-chrysanthemate	---	---	0.17
l-cis-Cineronyl-l-trans-chrysanthemate	---	---	0.12
Isohydropyrethrin I *	2.0	0.5	1.0
Isohydropyrethrin II *	Non-toxic at level used		Non-toxic at level used
Isohydrocinerin I*	3.6	0.35	0.56
Isohydrocinerin II *	Non-toxic at level used		Non-toxic at level used
Tetrahydropyrethrin I*	>16	0.06	< 0.06
Dihydrocinerin I*		0.08	---

*Hydrogenation of the side chain reduces toxicity appreciably and destroys the characteristic "knockdown" effect.

(2) Relative toxicity of pyrethrins vs. several insect species; as variously reported:

Component	Form	Remarks	Other	
Pyrethrin I	H_2O, suspension spray	1.25 times as toxic as II	(Blattella germanica) LC_{50} 24 hr { I = 10 mg/l; II = 12.5 mg/l	1207
Pyrethrin I	Kerosene spray	1.3 times as toxic as II	(Musca domestica) LC_{50} { I = 65 mg/100 cc; II = 85 mg/100 cc	1208
Pyrethrin I	H_2O spray + saponin	10 times as toxic as II	(Aphis rumicis) LC_{50} 24 hr { I = 1 mg/100 cc; II = 10 mg/100 cc	3058
Pyrethrin I	In miscible oil	Equal in toxicity to II	(Aphis rumicis)	1431
Pyrethrin I	In heavy mineral oil + H_2O	Equal in toxicity to II	(Tribolium castaneum)	2127
Pyrethrin I	In alcohol solution + H_2O	Many times more toxic than II	(Tribolium castaneum)	2127
Pyrethrin I	In acetone solution + H_2O	ca equal in toxicity (I & II)	(Musca domestica) Topical & Sprays	1431
Pyrethrin I	Kerosene spray	Yielded in 24 hrs twice the mortality given by II	(Musca domestica)	3005
Pyrethrin II	Kerosene spray	10 min. "knockdown" 3.5 times that of I	(Musca domestica)	3005
Pyrethrin I	Kerosene solution	Slightly greater mortality than with II	(Periplaneta americana) { Topical LC_{50} 24 hr; I = 1.0 mg/l; II = 1.5 mg/l	2178
Pyrethrin II	Kerosene solution	More rapid "knockdown" than I	(P. americana) { 50% "KD" 30 min. II = 1.0 mg/l; I = 1.5 mg/l	2178
Pyrethrolone; Chrysanthemum-mono-carboxylic acid; Chrysanthemum dicarboxylic acid		Non-toxic at 0.2 g/100 cc sprays, H_2O + saponin	(Aphis rumicis)	3058
Pyrethrin I	(Topical Administration)	2.6 times as toxic as II	(Phaedon cochlearis)	3227
Cinerin I	(Topical Administration)	2.5 times as toxic as II		
Pyrethrolone; Cinerolone	esterified with chrysanthemic acid (monocarboxylic)	4.3; 4.0 times as toxic as pyrethric acid ester vs. Musca as are kerosene sprays.		1147

(3) Toxicity of synthetic "pyrethroids" vs. Musca domestica; after [Ref. 2231] quoting [Refs.: 1148, 1161, 1159, 1162, 2752] :



R	R'	R''	Configuration (Acid)	Relative Toxicity Pyrethrins = 1
$CH_2CH=CHCH_3$	CH_3	H	d-trans	1.48
$CH_2CH=CH_2$	"	"	d-trans	6.64
$CH_2CH_2CH_2CH_3$	"	"	d-trans	0.17
$CH_2C(CH_3)=CH_2$	"	"	d-trans	3.46
$CH_2CH=C(CH_3)_2$	"	"	d-trans	0.21
$CH_2CH_2CH=CH_2$	"	"	d-trans	0.61
$CH_2C=CHCH=CH$ (O)	"	"	d-trans	1.92
$CH_2C=CHCH=CH$ (O)	"	"	dl-cis-trans	1.11
$CH_2CH=CHCH_3$	"	"	dl-cis	0.38
$CH_2CH=CHCH_3$	"	"	dl-trans	0.40
$CH_2CH=CH_2$	"	"	dl-cis	1.8
$CH_2CH=CH_2$	"	"	dl-trans	1.81
$CH_2C=CCH_3$	"	"	dl-cis-trans	0.73
$CH_2CCl=CH_2$	"	"	dl-cis-trans	1.56
$CH=CH=CHCl$	"	"	dl-cis-trans	1.42
$CH_2CH=CClCH_3$	"	"	dl-cis-trans	0.21
CH_3	"	"	dl-cis-trans	0.40
CH_2CH_2	"	"	dl-cis-trans	0.94
$CH_2CH=CH$	"	"	dl-cis-trans	3.24
$CH_2CH=CH$	"	$CH_2CH=CH_2$	dl-cis-trans	0.41
$CH_2CH=CH$	C_6H_5	H	dl-cis-trans	0.43

Summary: dl-trans esters slightly more toxic than d-cis-trans esters.
l-trans esters much less effective.
l-cis esters probably inactive.

acetopropyl alcohol, dextrose, levulose, mannose, phenol, carvacrol, salicyl aldehyde, hydroquinone, monomethyl ether, thymol, orcinol monomethyl ether, pyrocatechol, isoeugenol, o-allyl vanillin, benzyl alcohol, triphenyl carbinol, phenylethyl alcohol, p-methoxybenzyl alcohol, benzhydrol, o-hydroxybenzyl alcohol, methylsantonic acid, hydroxycamphor, hydroxymethylene camphor, cholestrin, methyl mercaptan, thiophenol, ethyl-, allyl-, benzyl- and β-phenylethyl-amines, aniline, p-nitroaniline and o-methoxyaniline.

The following had slight "knockdown" action:

esters of guiacol, eugenol, vanillin, piperonyl alcohol, allylphenyl carbinol, p-isopropylbenzyl alcohol, phenylpropyl alcohol, methyl styryl carbinol, cinnamic alcohol, citronellol, linalool, menthol, geraniol, methyl cyclohexanol, α-terpineol, borneol, sabinol, phytol, benzoin, benzoyl alcohol and dimethyl hexenelone.

The following proved inactive:

chrysanthemic acid esters of: 3-Methyl,- 3-phenyl-, 2,3-dimethyl-, 3-methyl-2-carbonester, 3-methyl-2-allyl-2-allyl-2-carbonester, 3-methyl-2-propenyl, cyclopentenolones and cyclopentanolones, 3-methyl-2-allyl- and 3-methyl-5-allyl-cyclopentenolones, styryl cyclopentenolone, 3-styryl-cyclopentadiene dicarbonester and benzal cyclopentanolone.

(c) Esterification of chrysanthemic acid with the following yielded compounds less than 0.06 as effective as natural pyrethrins vs. Musca: Furfuryl alcohol, αα-dimethyl phenethyl alcohol, 1-(p-tolyl)-ethanol, p-cresol, hydroquinone diester, p-methoxyphenol and 8-quinolinol. 2286

(d) The following are non-toxic to Musca: p-Chlorophenyl dl-cis- and dl-trans-chrysanthemate, p-chlorophenacyl-, p-chlorophenethyl- and 2,2,2-trichloroethyl-dl-trans-chrysanthemate; p-chlorobenzyl dl-trans-chrysanthemate shows moderate toxicity vs. Musca. 1399

(9) Other important structural considerations:

(a) Position of side-chain unsaturation, for example, d-trans-chrysanthemate of 2-but-2' -enyl-3-methyl-cyclopent-2-en-4-ol-1-one proved 2.4 times as toxic for Musca as 2-but-3' -enyl cyclopentenolone. 1148

(b) Introduction of a triple bond, as in but-2-ynyl side-chain yielded no change in toxicity. 1159

(c) Chlorination of cinerolone side-chains brought decrease in toxicity of dl-cis-trans chrysanthemates to ½ that of the unchlorinated analogues. 1159

(d) Esterification of cinerin I with chrysanthemic acid at position 5, rather than 4, in the cyclopentenolone ring yielded a product ca. 0.12 as toxic as cinerin I. 1890

(10) In summation, it may be said that virtually all structural modification of the pyrethrin or cinerin molecule, whether in the alcoholic or acidic component or moiety, degrades the toxicity and modifies the "knockdown" capacity.

c) Quantitative toxicity; insects:

(1) For several insect species as determined by one investigator: 2219

Insect	Route	Dosage (μg/g) For Mortality % Shown of Pyrethrins I, II								
		0%			50%			100%		
		♂	♂♀	♀	♂	♂♀	♀	♂	♂♀	♀
Anasa tristis	Topical	-	2	-	-	7	-	-	26	-
Anasa tristis	Injection	-	4	-	-	10	-	-	25	-
Bombyx mori (larva)	Topical	-	-	-	-	-	-	-	< 0.4	-
Ceratomia catalpae (larva)	Topical	-	0.7	-	-	2	-	-	6	-
Ceratomia catalpae	Injection	-	1	-	-	4	-	-	6	-
Oncopeltus fasciatus	Topical	-	3	-	-	8	-	-	28	-
Periplaneta americana	Topical	2	-	6	4	-	9	6	-	12
Periplaneta americana	Oral	8	-	18	14	-	29	20	-	40
Periplaneta americana	Injection (blood)	1	-	5	3	-	8	6	-	11
Popillia japonica	Topical	-	10	-	-	40	-	-	130	-
Popillia japonica	Injection	-	10	-	-	40	-	-	110	-
Tenebrio molitor	Topical	-	25	-	-	35	-	-	100	-

(2) Observations by various investigators:

Insect	Route	Dose	Dosage	Remarks	
Aedes aegypti (adult ♂)	Contact Spray	LD_{50}	0.5 (.5-1.0) μg/g	As 0.1% w/v solution.	693
Aedes aegypti (" ♀)	Contact Spray	LD_{50}	1.0 (1.0-1.5) μg/g	As 0.1% w/v solution.	693
Chaoborus astictopus (winter larva)	Medium	LC_{100}	0.33 ppm	Solution sans wetting agent.	768
Chaoborus astictopus (")	Medium	LC_{99}	0.2 ppm	"	768
Chaoborus astictopus (")	Medium	LC_{66}	0.033 ppm	"	768
Chaoborus astictopus (")	Medium	LC_{100}	0.2 ppm	Solution + Na lauryl SO_4 wetting agent.	768
Chaoborus astictopus (")	Medium	LC_{93}	0.1 ppm	"	768
Chaoborus astictopus (")	Medium	LC_{63}	0.033 ppm	"	768
Chaoborus astictopus (")	Medium	LC_{36}	0.016 ppm	"	768
Agrotis orthogonia (larva)	Spray	$LDeposit_{50}$	8.2 μg/cm²		350
Peristoneura fumiferana (larva)	Spray	$LDeposit_{50}$	0.05 μg/cm²		350
Culex lectularius (adult)	Contact Spray	LD_{50}	0.02 μg/insect		413
Culex lectularius (")	Contact Spray	LD_{50}	5 μg/g		413

(2) Observations by various investigators (Continued):

Insect	Route	Dose	Dosage	Remarks	
Cimex lectularius (adult)	Contact Spray	LD_{50}	0.012 μg/insect	Pyrethrins + 2% isobutyl undecylenamide.	413
Cimex lectularius (")	Contact Spray	LD_{50}	3 μg/g	"	413
Apis mellifera (")	or	LD_{50}	0.5 μg/g	At 20°C.	296
Apis mellifera (")	or	LD_{50}	5.0 μg/g	At 34.5°C.	296
Heliothis ononis (larva)	Spray	$LDeposit_{50}$	4.0 μg/cm²		350
Pediculus humanus corporis	Contact Spray	LD_{50}	0.085 μg/insect		413
Pediculus humanus corporis	Contact Spray	LD_{50}	42 μg/g		413
Pediculus humanus corporis	Contact Spray	LD_{50}	0.007 μg/insect	Pyrethrins + 2% isobutyl undecylenamide.	413
Pediculus humanus corporis	Contact Spray	LD_{50}	3.5 μg/g	"	413
Pediculus humanus corporis	Residue	LDeposit	6 μg/cm²	On flannel as 50% in oil.	414
Pediculus humanus corporis	Residue	LDeposit	4.5 μg/cm²	" 10% " .	414
Pediculus humanus corporis	Residue	LDeposit	31 μg/cm²	" in volatile solvent.	414
Pediculus humanus corporis	Contact Spray	LC_{50} (%)	34%	Commercial; 0.44% pyrethrins in oil at 0.36 mg/cm².	414
Pediculus humanus corporis	Contact Spray	LC_{50} %	3%	Pyrethrins + 2% isobutyl undecylenamide at 0.36 mg/cm².	414
Fannia canicularis (adult)	Topical	LD_{50} 24 hr	♀0.24,♂0.44 μg/fly	In acetone; measured drop test.	1981
Phaedon cochleariae (adult)	Contact Spray	LC_{50} w/v	.00037%	Application as aqueous sprays + 0.1% sulfonated lorol, 10% acetone in the Potter tower.	935
Phaedon cochleariae (")	Contact Spray	LC_{50} w/v	.000305%		935
Phaedon cochleariae (")	Contact Spray	LC_{50} w/v	.000324%		935
Macrosiphum solanifolii (adult♀♀)	Contact Spray	LC_{50} w/v	.000541%		935
Macrosiphum solanifolii (")	Contact Spray	LC_{50} w/v	.000704%	"	935
Macrosiphum solanifolii (")	Contact Spray	LC_{50} w/v	.00034%		935
Oryzaephilus surinamensis (adult)	Contact Spray	LC_{50} w/v	.00552%		935
Oryzaephilus surinamensis (")	Contact Spray	LC_{50} w/v	.00789%	"	935
Oryzaephilus surinamensis (")	Contact Spray	LC_{50} w/v	.00537%		935
Plutella maculipennis (larva, last instar)	Contact Spray	LC_{50} w/v	.00899%		935
Plutella maculipennis (")	Contact Spray	LC_{50} w/v	.00346%	"	935
Plutella maculipennis (")	Contact Spray	LC_{50} w/v	.005754%		935
Pliophila casei	Aerosol	LC_{98} 24 hr	50 mg/960ft³	In dichlorodifluoromethane + sesame oil.	243
Pliophila casei	Aerosol	LC_{66} 24 hr	25 mg/960ft³		243
Anopheles quadrimaculatus (larva)	Medium	MLC_{100}	0.1 ppm	78% kill at 0.05 ppm.	2020
Musca domestica (adult)	Contact Spray	LC_{50} 24 hr	1.2 ±.14 mg/cc	Standard pyrethrins as acetone: kerosene spray 1:1.	1164
Musca domestica (" ♂)	Contact Spray	LD_{50}	31.0 (30-35) μg/g	As 2% w/v pyrethrins in 50/50 odorless distillate + benzene.	693
Musca domestica (" ♀)	Contact Spray	LD_{50}	38.0 μg/g	"	693
Musca domestica (adult)	Topical	LD_{50} 24 hr	1.0 μg/fly	Laboratory (non DDT-R) strain, measured drop method.	78
Musca domestica (")	Topical	LD_{50} 24 hr	1.0 μg/fly	Bellflower (DDT-R) strain, measured drop method.	78
Musca domestica (")	Topical	LD_{50} 24 hr	2.0 μg/fly	San José (DDT-R) strain, measured drop method.	78
Musca domestica (")	Topical	LD_{50} 24 hr	2.0 μg/fly	Ontario (DDT-R) strain, measured drop method.	78
Musca domestica (")	Topical	LD_{50} 24 hr	2.0 μg/fly	Riverside (DDT-R) strain, measured drop method.	78
Musca domestica (")	Topical	LD_{50} 24 hr	88.1 μg/g	DDT-I R-strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (")	Topical	LD_{50} 24 hr	74.8 μg/g	DDT-W, R-strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (")	Topical	LD_{50} 24 hr	65.8 μg/g	DDT-III, R-strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (")	Topical	LD_{50} 24 hr	80.2 μg/g	Methoxy-I, R-strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (")	Topical	LD_{50} 24 hr	62.5 μg/g	Lindane-I, R-strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (" ")	Topical	LD_{50} 24 hr	89.9 μg/g	Multi-I, multi-R strain; pyrethrins + piperonyl butoxide 1:10.	373

(2) Observations by various investigators (Continued):

Insect	Route	Dose	Dosage	Remarks	
Musca domestica (adult)	Topical	LD_{50} 24 hr	56.8 μg/g	Lab-I, non-R strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (")	Topical	LD_{50} 24 hr	49.1 μg/g	Lab-II, non-R strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (")	Topical	LD_{50} 24 hr	258.7 μg/g	Pyro-I*, pyrethrin-R strain; pyrethrins + piperonyl butoxide 1:10.	373
Musca domestica (")	Topical	LD_{50} 24 hr	81.6 μg/g	Multi-III, multi-R strain; pyrethrins + piperonyl butoxide 1:10.	373
*21 generations of selection by exposure of larvae and adults to pyrethrins; origin of strain: Lab I.					
Chironomus spp. (larvae)	Medium	LC_{100} 90-93 hrs	12 ppm w/w	0.9% pyrethrins as pyrethrum powder.	979
Lygus {atriflavus, elisus, hesperus} (nymph)	Dust	$LDeposit_{50}ca$	25.9 mg/100cm²	A dust c 2% pyrethrins and talc.	1010
Lygus " (adult)	Dust	$LDeposit_{50}ca$	>11 mg/100cm²	"	1010
Lygus " (nymph)	Dust	$LDeposit_{100}ca$	>47.5 mg/100cm²	"	1010
Lygus " (adult)	Dust	$LDeposit_{100}ca$	47.5 mg/100cm²	"	1010
Lygus " (adult, nymph)	Dust	$LDeposit_{97}$	31.8 mg/100cm²	Pyrethrum + cube.	1010
Musca domestica (adult)	Topical	LD_{50} 24 hr	1.0 μg/fly	At 60°F; Lab strain DDT-non-R strain.	371
Musca domestica (adult)	Topical	LD_{50} 24 hr	0.94 μg/fly	At 60°F; Bellflower, DDT-R strain.	371
Musca domestica (adult)	Topical	LD_{50} 24 hr	1.6 μg/fly	At 60°F; Pollard, DDT-R strain.	371
Musca domestica (adult)	Topical	LD_{50} 24 hr	1.13 μg/fly	Piperonyl butoxide-pyrethrins 10:1.	371
Aceratogallia sanguinolenta	Dipping	LC_{100}	ca. 2604 g/100cc H_2O	As a suspension; exposure 30 seconds.	3239

d) Comparative toxicity of pyrethrins and other compounds for insects:

(1) Vs. Musca domestica; topical application by measured drop method: 78

Strain	LD_{50} (μg/fly) 24 Hrs For						
	Pyrethrins	DDT	DDD	Methoxychlor	Toxaphene®	Lindane	Heptachlor
Bellflower (DDT-R)	1	10	20	1	0.6	0.08	0.06
San José (")	2	0.7	-	0.3	0.4	0.05	0.07
Ontario (")	2	0.5	-	0.3	0.5	0.05	0.07
Riverside (")	2	0.5	-	0.3	0.5	0.06	0.07
Laboratory (DDT-non-R)	1	0.02	0.1	0.07	0.2	0.01	0.03

(2) Vs. Musca domestica and Aëdes aegypti adults by spraying with pyrethrins 0.1% w/v for Aëdes and 2.0% w/v for Musca; DDT and BHC .3%; all in odorless distillate + benzene (50:50). 693

Insecticide	LD_{50} (μg/g) For			
	Musca		Aëdes	
	♂	♀	♂	♀
Pyrethrins	31.0 (30 - 35)	38.0	0.5 (0.5 - 1.0)	1.0 (1.0 - 1.5)
DDT	6.0 (5.5 - 7.0)	9.0(9.3-10.5)	5.5 (4.5 - 5.5)	8.0 (7.5 - 8.5)
BHC	2.0	3.0	3.0	3.5

(3) Vs. Musca domestica; as acetone + kerosene (1:1) sprays: 1164

Insecticide	Mean Concentration For 50% Mortality (mg/cc) 24 Hrs	Relative Toxicity At LC_{50}
Pyrethrins (standard)	1.2 ± 0.14	0.43 ± 0.06
Methyl tetraphosphate	0.52 ± 0.05	1.0 (STANDARD)
Tetraethyl pyrophosphate	0.095 ± 0.01	5.5 ± 0.7
Malathion	0.03 ± 0.003	17.0 ± 2.0

(4) Pyrethrins and allethrin vs. various insects; spraying tests, with insects held at 17° - 20°C after treatment; treated in Potter tower; aqueous sprays 0.1% lorol + 10% acetone: 935
I = (±)-3-methyl-2-allyl-cyclopent-2-en-4-ol-1-one esterified with (+)-trans-chrysanthemum monocarboxylic acid (natural acid) = pyrethrin.

II = +, (±)-allylrethronyl (+)-trans-chrysanthemate.
III = +, (±)-allylrethronyl (±)-cis-trans chrysanthemate.

(a)

** Insect	LD_{50}% (w/v) I	II
Plutella maculipennis (larva, last instar)	0.00899;0.00346	0.00251;0.00241
Macrosiphum solanifolii (adult, apterous ♀♀)	.000541;.000704	.00272; .0092
Phaedon cochleariae (adult)	.000371;.000305	.000662;.002009
Oryzaephilus surinamensis (adult)	.00552;.00789	.0112;.02607

**Data of various experiments performed at different times.

(b) Data from insects all sprayed on the occasion of the same experiment: A = natural pyrethrins, B = ± -allylrethronyl (+)-trans-chrysanthemate, C = ± -allylrethronyl (±)-cis-trans-chrysanthemate.

Insect	LD_{50}% (w/v) A	B	C
Plutella maculipennis (larva)	0.005754	0.001415	0.003162
Macrosiphum solanifolii (adult, apterous ♀♀)	0.00034	0.00275	0.00589
Phaedon cochleariae (adult)	0.000324	0.000813	0.001662
Oryzaephilus surinamensis (adult)	0.00537	0.01122	0.01318

(c) Relative potencies:

	Vs. Plutella	Vs. Macrosiphum	Vs. Phaedon	Vs. Oryzaephilus
A	1000	1000	1000	1000
B	3980	123	399	479
C	1820	58	200	406

(5) Vs. Pediculus humanus corporis and Cimex lectularius; spraying tests, with toxicants in P31 oil, the solutions sprayed at 0.36 mg/cm², a rate at which the oil carrier is harmless; contact spraying: 413

Insecticide	Pediculus LC_{50}(%)	Cimex LC_{50}(%)	LD_{50} Pediculus µg/insect	Pediculus µg/g	Cimex µg/insect	Cimex µg/g
Pyrethrins	0.47	0.045	0.085	42	0.02	5
Pyrethrins + 2% isobutyl undecylenamide	0.038	0.026	0.007	3.5	0.012	3
Lindane	0.016	0.051	0.003	1.5	0.023	6
DDT	0.030	0.56	0.054	27.0	0.25	63
Lethane® 384	1.5	4.0	0.27	135	1.8	450
Lethane® special	2.4	12.5	-	-	-	-
Thanite®	3.2	75.0	-	-	-	-
Lauryl thiocyanate	6.0	19.5	-	-	-	-
Bis-ethyl xanthogen	6.2	75.0	-	-	-	-
Lethane® 60	8.1	32.0	-	-	-	-
Benzyl benzoate	21.0	75.0	-	-	-	-

(6) As contact toxicants for larvae of several insect species; contact sprays: 350

Insecticide	Lethal $Deposit_{50}$ (µg/cm²) For Choristoneura fumiferana	Heliothis ononis	Agrotis orthogonia
Pyrethrins	0.05	4.0	8.2
DDT	0.3	7.0	80.0
Lindane	1.9	23.0	5.5
Chlordane	140.0	Ineffective	18.0
DNOC	4.0	16.0	7.5
Nicotine	42.0	400.0	Ineffective

(7) Vs. various insect species: [Ref. 2219]

Insect	Route	Dosage (µg/g) To Yield Mortality (%) Indicated: Pyrethrins I, II 50%	Pyrethrins I, II 100%	Lethane 384 50%	Lethane 384 100%	Sodium arsenate 50%	Sodium arsenate 100%	Derris Resins* 50%	Derris Resins* 100%	Nicotine 50%	Nicotine 100%
Anasa tristis	Topical	.007	.026	-	-	-	-	-	-	.35	1.25
"	Injection	.01	.025	-	-	.02	.04	.010	.025	.20	.35
Bombyx mori (larva)	Topical	-	<.0004	-	-	-	-	-	<.0007	.004	.008
"	Injection	-	-	-	-	-	-	-	-	.003	.007
Ceratomia catalpae (larva)	Topical	.002	.006	-	-	-	-	.002	.005	.10	.20
"	Injection	.004	.006	-	-	.02	.03	.004	.006	.08	.15
Oncopeltus fasciatus	Topical	.008	.028	0.40	0.75	-	-	.025	.06	.19	.45
"	Injection	-	-	-	-	-	-	-	-	-	-

(7) Vs. various insect species (Continued):

Insect	Route	Dosage (μg/g) To Yield Mortality (%) Indicated									
		Pyrethrins I, II		Lethane 384		Sodium arsenate		Derris Resins*		Nicotine	
		50%	100%	50%	100%	50%	100%	50%	100%	50%	100%
Periplaneta americana	Topical	.0065	.012	0.96	2.3	.25	1.30	-	-	.65	1.3
"	Injection	.006	.011	0.17	0.40	.045	.07	.007	.013	.1	.2
Popillia japonica	Topical	.04	.13	.80	1.70	.85	1.70	.025	.06	.65	1.0
"	Injection	.04	.11	.30	.09	.05	.10	.04	.11	.40	.90
Tenebrio molitor	Topical	.035	.1	.85	1.60	-	-	.019	.075	3.2	4.4
"	Injection	-	-	-	-	-	-	-	-	-	-

*For dosage as rotenone per se use 25% of the given value.

(8) Vs. Chaoborus astictopus (overwintering larvae): 768

Insecticide	PPM	Mortality (%)
Pyrethrum (without wetting agent)	0.33	100
	0.2	99
	0.033	66
Pyrethrum (+ Na lauryl sulfate wetting agent)	0.2	100
	0.1	93
	0.033	63
	0.016	36
Derris (as rotenone)	1.0	98.3
	.5	97.5
Phenothiazine	0.33	100
1-Phenylbenzothiazole (in oil + CCl_4)	0.33	100
	0.2	66

e) Other toxicity tabulations:

(1) Relative susceptibility of diverse insects to pyrethrum sprays (in Turkey Red Oil) and dusts (in talc); pyrethrum deposits at 0.38 μg/cm²: 2393

Insect	Spray % Mortality	Dust % Mortality
Bombyx mori	100	100
Agelastica alni	100	100
Vanessa polychloros	100	100
Euproctis chrysorrhoea	100	--
Athalia spinarum	100	--
Dendrolimus pini	100	96
Vanessa io	100	90
Vanessa urticae	100	--
Smerinthus ocellata	100	--
Agrotis spp.	5	10
Lymantria monacha	0	--
Stilpnotia salicis	0	0
Carpocapsa pomonella	0	0
Oryctes nasicornis	0	--
Melolontha spp.	0	--
Myzus persicae	0	--

(2) Stage of development and susceptibility to pyrethrin sprays; larvae of Stilpnotia salicis: 1822

Instar	% Mortality	Time For Death
I	100	12 hrs.
II	100	16 hrs.
III	100	3 days
IV	65	4 days
V	15	5 days
VI	0	5 days

(3) Pyrethrins and other compounds vs. Musca domestica (adult), Fannia canicularis (3 day laboratory reared adults; av. wgt. ♂ = 6.89 mg; ♀ = 7.35 mg). 1981

Insecticide	Approximate LD_{50} 24 Hrs (μg/insect)		
	Musca domestica ♀	Fannia canicularis	
		♀	♂
Pyrethrins	1.0	0.24	0.44
DDT	0.033	2.80	1.30
Methoxychlor	0.068	0.14	0.12
Lindane	0.01	0.76	0.39

(3) Pyrethrins and other compounds vs. Musca domestica (adult), Fannia canicularis (3 day laboratory reared adults; av. wgt. ♂ = 6.89 mg; ♀ = 7.35 mg) (Continued):

Insecticide	Approximate LD_{50} 24 Hrs (μg/insect)		
	Musca domestica ♀	Fannia canicularis	
		♀	♂
Dieldrin	0.031	0.003	0.0026
Malathion	0.56	0.1	0.06
Diazinon	0.092	0.098	0.054
Chlorthion®	0.33	0.035	0.022

(4) Pyrethrins and chlorinated hydrocarbons as space sprays vs. Musca domestica (adult), applied by Campbell Turntable Method; sprays made up in kerosene; variations reflecting differences in resistance and susceptibility of various fly "populations": 1152

Insecticide	Concentration (mg/cc)	"KD" 25 min (%)	Mean Mortality 24 Hrs.	LC_{50} (mg/cc)	Relative Toxicity Compared To Pyrethrins	Relative Toxicity Compared To Chlordane (tech)
Pyrethrins	8.0	100	82			
	4.0	100	58;63	3.32 ± 0.25;		
	2.0	100	71;36;26	2.83 ± 0.36	1.0	-
	1.0	100	32;17;13	1.37 ± 0.16		
Aldrin	0.25	7;5	85;82			
	0.125	8;6	45;51	0.131 ± 0.01	25;22	4.0
	0.063	9;3	15;15	0.129 ± 0.017		
Chlordane (tech) Sample A	1.0	8;10	99;84			
	0.5	7;3	74;51	0.33 ± 0.04	4.2;6.4	1.0
	0.25	11;6	33;12	0.52 ± 0.039		
Chlordane (tech) Sample B	1.0	9	93			
	0.5	11	70	0.39 ± .05	3.5	-
	0.25	6	20			
Chlordane (crystalline)	1.0	9	66			
	0.5	9	28	0.743 ± 0.055	4.5	0.7
	0.25	6	11			
Dieldrin	0.25	5	98			
	0.125	1	74	0.088 ± 0.011	32	5.9
	0.063	2	27			
Heptachlor	0.5	14;-	100 -			
	0.25	8;4	100 93	0.114 ± 0.009	ca 28	4.0;4.4 (73% Mortality Level)
	0.125	7;5	73 45			
	0.063	- 7	— 17			

(5) Susceptibility of Periplaneta americana and Blattella germanica to pyrethrin sprays, directly applied to dorsum; concentration = 5 mg pyrethrins per cc kerosene: 2177

Stage	Spray Deposit (μg/cm^2)	% Mortality Of Periplaneta	% Mortality Of Blattella
Adult ♀	960	--	100
" "	560	100	72
" "	280	56	27
Large Nymphs	960	--	87
"	560	77	60
"	280	52	25

"KD" more rapid for Periplaneta; Blattella more resistant to killing effect.

Pyrethrum (0.9% pyrethrins) as a dust at 0.81 mg/cm^2 for Blattella germanica, topical application gave 14% mortality in 24 hours; 100% mortality in 96 hours; average survival time ♂ = 7.8 hours, ♀ = 49.3 hours. Pyrethrum 25% + pyrophyllite 75% topical application gave 81% kill in 24 hours, 86% kill in 96 hours; average survival time ♂ = 3.5 hours, ♀ = 26.6 hours. 777

(6) Relative susceptibility of Stomoxys calcitrans and Musca domestica to pyrethrum and kerosene sprays; used as OCI (Official Control Insecticide of the National Ass'n. of Insecticide and Disinfectant Manufacturers) as a mist, in vault (28.5 m^3) at 27°C; exposure 10 minutes: 887

Insect	Dose (cc. OCI)	Fraction of LC_{50} For Musca domestica	Result
Stomoxys calcitrans	5.6	1/10	100% initial torpor; 100% kill.
"	2.8	1/20	" " " ; " " .
"	1.12	1/50	97% " " ; " " .
"	.56	1/100	<75% " " ; 93% " .
"	.56 + 9.4 cc refined kerosene	1/100	<75% " " ; 88% " .
"	5.6cc refined kerosene	--	10% disabled
Musca domestica	55.90	1/1	98% initial torpor; 51% kill.

(7) Vs. Aceratogallia sanguinolenta; pyrethrum as a powder (0.92% total pyrethrins); by immersion for 30 seconds in water-suspension; dosage and net mortality %: 3239

g/100 cc H_2O	Net Mortality %
1.0416	100
.521	100
.2604	100*
.1302	98.9
.0651	89.3
.0326	65.8
.0163	43.2
.0082	22.0
.0041	17.7
.0021	3.5

(* = to 4 lbs. of 0.5% flowers/100 gal.)

(8) Effect on Phormia regina dipped in (A) = solutions of a commercial insecticide: Esters of mannitan + coconut oil fatty acids + pyrethrins 1% and (B) = control using foregoing esters without pyrethrins; insects of various ages: 70

(Age of flies —)	3 - 12 hrs		<5 minutes	36 - 48 Hrs	<5 minutes
Material dilution	% Kill 48 Hrs. After Dipping for 15 Seconds in				
	A	B	A	A	A
1:100	100	83	--	--	--
1:200	100	100	--	--	--
1:400	100	67	--	100	33
1:800	100	33	10	89	11
1:1600	100	50	10	89	11
1:3200	88	17	0	33	0
1:6400	33	0	0	0	11
1:12,800	14	17	0	--	--
1:25,600	14	17	--	--	--

(9) Effects of atmospheric environment (temperature, relative humidity) before and after treatment; adult Tribolium castaneum sprayed with pyrethrins in aqueous medium, with or without terpineol; hot = 80°F, cold = 60°F; at 80° relative humidity = 60%; at 60° R. H. = 48 - 56%: 2532

Treatment of Insects		Terpineol	LD_{50} As % Total Pyrethrins (w/v)	
Before Spraying	After Spraying	(present (+) / absent (-))	Experiment I	Experiment II
Cold	Cold	(-)	0.0044	0.0031
Hot	Cold	(-)	.0049	.0033
Cold	Hot	(-)	.0092	.0098
Hot	Hot	(-)	.0116	.0156
Cold	Cold	(+)	.0027	.0018
Hot	Cold	(+)	.0045	.0018
Cold	Hot	(+)	.0122	.0110
Hot	Hot	(+)	.0198	.0125

Insecticide	Medium	Potency Under Cold Storage (As Proportion Of Potency Under Hot Storage)		
		Cold Before Spraying	Cold After Spraying	Cold Before, Cold After
Pyrethrins	aqueous	1.19;1.31	2.25;3.84	2.67;5.01
Pyrethrins + terpineol	"	1.62;1.07	4.45;6.49	7.21;6.9
Lauryl thiocyanate	"	0.98	1.47	1.43
Nicotine	"	0.99	1.24	1.23
DNOC	ethylene glycol	--	--	1.46
DDT	Wakefield oil	--	--	2.61
Wakefield half-white oil		--	--	0.87

f) Toxicity for beneficial insects: (Also see Bees and Insecticides)

(1) Safe for Apis mellifera within a few minutes of application. 3099

(2) As a spray of pyrethrum with 6% extractives, at 1 part to 400 parts water, the average kill of Hippodamia convergens larvae (1st instar) = 8% (3 - 13%); of eggs = 3% (0 - 9%). 1450

g) Pharmacological, pharmacodynamic, physiological, etc.; for insects:

(1) Mode of action!

(a) Primarily contact insecticides; modest stomach toxicity. 2231,353,2815

(b) Manifestations following oral intake; various insects: 1059,757,212

Insect	Sequelae Of Oral Intake	
Agrotis segetum	Regurgitation, spasm, quiescence → recovery.	3207
Pieris brassicae	" "	3207
Porthetria dispar	" "	3207
Locusta migratoria migratorioides	" "	3207
Blatta orientalis	Symptoms disappearing after 12 hours.	1545
Prodenia eridania	No effects following oral intake; susceptible to contact action.	3348
Apis mellifera	Highly toxic on oral intake.	296
Mosquito larvae	Little or no pyrethrins in tissue, digestive tract or feces 6 - 24 hrs. after intake.	353

(c) Manifestations of contact action:

	Sequelae Of Contact Exposure	
Tenebrio molitor Periplaneta americana	Applied as droplets 0.15% pyrethrins in kerosene to antennae, cerci, legs, head, spiracles abdomen, thorax: Gave toxic symptoms arising after application to neck and intersegmental areas in ½ the time required after application on highly sclerotized areas.	2405
Macrodactylus subspinosus	— 1 drop pyrethrin on tarsus brought rapid death.	1429
Glossina morsitans	—Application to pulvilli gave paralysis in 2 seconds or more.	2535
Blatta orientalis	—Topical application as 25% pyrethrum dust: For 1.5 minutes no reaction; after 2 minutes sudden, intense excitement then paralysis first of the metathoracic legs followed by spread of paralysis to other members rendering the insect helpless in 8 minutes. Effects localized on restricted local application; ½ or more of surface must be dusted to ensure total paralysis in 12 hours. Application directly to tracheae proved ineffective.	1545
Periplaneta americana	Applied as dry powder in spiracles: Brought symptoms (legs) similar to those noted after giving kerosene solutions of pyrethrins by injection.	2713

(d) Rate of penetration * (measured as approximate time for paralysis) of pyrethrins in various solvents through the cuticle of Rhodnius prolixus after topical application; 2% solutions of pyrethrins: 3297

Solvent	B.P. (°C)	Time For Paralysis To Occur
Hexane	-	1.25 hrs.
Heptane	-	1.5 hrs.
White Spirit	150-190	2 hrs.
White Oil	265-365	5 hrs.
White Oil	310-390	10 hrs.
Odorless Distillate	200-260	4 hrs.
A 12 Oil	260-360	6 hrs.
P 31 (refined heavy paraffin oil)	320	6-28 hrs.
Oleic acid	-	4.5 hrs.
Olive Oil	-	36-96 hrs.
Castor Oil	-	36-96 hrs.
Sesame Oil	-	36-96 hrs.

*Onset of symptoms correlated with boiling point of solvent; vegetable oil solvents yield slow response; pretreatment with petroleum ether speeds the onset of symptoms. Response also associated with cuticular thickness: At 8 - 9 micra cuticle thickness 1.5 hrs; at 10 micra 2 hrs; at 18 micra 8 hours.

(e) Musca domestica and Aëdes aegypti flying through finely dispersed pyrethrin mists (sprays in odorless kerosene + benzene [50:50]) accumulate a large proportion of the dose on the wings. Addition of Sudan III shows penetration of wings directly, with later appearance in Malpighian tubules; the material may also be removed from wings in the grooming process and be transferred to and absorbed via the legs. 693

(2) Theories of toxic action of pyrethrins:

(a) By some the toxophoric portion of the pyrethrin molecule is thought to be at $-\underset{L}{C}=\underset{L}{C}-C\overset{O}{\leq}O-L$ where L = a lipid solubilizing group; the cyclopropane ring, methyl, dimethyl and allene groups are believed responsible for lipid solubility. 934 1933

(b) Another theory is based on cuticular permeability to pyrethrins with subsequent effects on tissue receptors controlling oxidative enzyme systems. Access of pyrethrins to insect interior facilitated (a postulate) by absorption and storage in epicuticular lipophilic layers. According to this theory, "pyrethrinization" consists of: I) Narcosis ("knockdown") phase, with block of oxidase action by absorption of pyrethrin to lipo-protein tissue complexes; II) death follows when a dispersant action brings on irreversible increase in phenol oxidase activity (through displacement of protective lipids) which leads to build-up of toxic metabolites in blood and tissues. Relative susceptibility and refractoriness of various insect species is laid to differences in cuticle make-up and internal factors associated with oxidase system stability. 1629 1627

(3) Age of insects and sensitivity to pyrethrins: (See some of the preceding tabulations in the part headed Toxicological).

(a) In the case of the tick Ornithodorus moubata (Argasidae) older larvae react more slowly to immersion in pyrethrin solutions than do young larvae. 2679

(b) Young Musca domestica adults are more rapidly paralyzed and less rapidly killed by sprays than older subjects. 2837, 70

(c) Vs. Loxostege sticticalis larvae pyrethrin dusts and sprays proved very effective in case of instars I, II, III; practically ineffective for instar V (there is a decrease of fat in the exoskeleton from 11.7% in instar III to 0.2% in instar V). 2471

(4) Site of application and "knockdown" effect:

(a) Application of 1 μg pyrethrins in kerosene to the thoracic dorsum of Musca domestica, Calliphora vomitoria gave 40% "knockdown" (on the average) in 13 minutes; application to mouth parts and thoracic spiracles gave 100% "knockdown" in 1 - 3 minutes; application of 0.01 μg in kerosene at the cervical membrane and inter-coxal regions gave instantaneous "knockdown". 3318

(5) Miscellaneous factors influencing penetration of the insect body by pyrethrins:

(a) Increase in relative humidity enhances penetration and hastens onset of symptoms in Lymantria monacha (larva) treated at 21°C with pyrethrum dusts. 329

(b) Solvents with high surface activity, such as alcohols, aldehydes, ethers and esters penetrate rapidly the cuticle cut from living insects; disaccharides and amino-acids (with little surface activity) penetrate not at all or slightly; fatty acids and paraffins do not enter. 329

(c) Mixtures of polar and apolar solvents (alcohol and paraffin oil) penetrate the cuticle of Calliphora erythrocephala (larva) with extreme rapidity (4 - 6 minutes) while neither one alone is effective within 1 hour. 1626

(d) Reported by some that the partition coefficient of a toxic material, such as pyrethrins, determines the rate at which it will leave an oily carrier to enter the insect cuticle. 3299

(6) General toxic effects of pyrethrins; symptoms, signs:

(a) Symptom sequence which followed the placing of a droplet on the dorsum of Protoparce sexta (larva): Insect normal for $\frac{1}{2}$ hour; last pair prolegs affected; paralysis of treated segment; regurgitation; insect rolled over and over for ca. 15 minutes; uncoordinated, violent movements for ca. 30 minutes; locomotion ceased; death in ca. 24 hours. A droplet on head: Beginning of response in 8 minutes. Injection into last abdominal segment: Intoxication in 2 minutes. 1429

(b) Rhodnius prolixus: Pyrethrin treatment leads to: I) incoordination of hind legs; II) all legs uncoordinated but walking possible; III) inability to walk, progressive extension of proboscis; IV) paralysis, which may continue 10 - 20 days, with the heart beating and continued gut movements. 3297

(c) Blattella germanica pyrethrin treated (oil solutions) showed: At ca. 2 second latent period; (dusts) 5.5 second latent period followed by intense excitement then submaximal activity with legs incompletely relaxed. 1632

(d) Thermobia domestica, recovering from sub-lethal pyrethrin exposure, reveals delayed effects: Appearance of discolored areas, sloughing of appendages, (legs, antennae, cerci, palpi, ovipositor) appearing as long as 19 weeks following exposure. 353

(7) Physiological action of pyrethrins:

(a) Effects on heart rate: in Corethra larvae: Progressively slowed. 1865
in Galleria mellonella: Slowed; diastole prolonged. 213
in Blatta orientalis: Heart stopped in systole. 3382
in Bombyx mori: Applied to heart at 0.007% in saline: Amplitude and frequency increased; at 0.01% in saline: Amplitude and frequency decreased followed by frequent and partly reversible cessation of beat. 1598
In the pyrethrinized Rhodnius heart beat may continue 10 - 20 days after complete paralysis. 3297

(8) Locus of action:

(a) Primary action is most probably on the central nervous system, with apparent blocking of nerve impulse transmission. Histopathological changes occur in the neurons. The evidence supporting this conclusion: I) A stimulating effect leading to paralysis and death; II) histopathological changes; III) accumulation and threshold concentration prior to action; IV) early loss of nerve responsiveness to electrical stimulation; V) selective penetration to nervous system; VI) effects on the action potential. 151,2713, 3278,1865, 777,1420, 2047,2600, 893

(b) In Periplaneta americana: 1632,2713
Application of pyrethrins to abdomen (nerve cord sectioned at 3rd abdominal segment): On legs no effect; abdominal twitching.
Applied to thorax: Twitches in the isolated legs.
Isolation of abdomen behind 3rd segment save for nerve cord: No prevention of pyrethrin action.
Applied to abdomen (nerve cord sectioned): Death.
Applied to cut end of isolated leg: Muscle fibrillation, slow contraction.
Applied to thoracic ganglion: Immediate paralysis of legs innervated thereby.
Applied to abdomen (nerve cord severed in ganglion): Leg paralysis posterior to the site of section.
Applied to leg: Stimulation of crural nerve yielded discharges at ever shorter intervals with contraction of leg followed by continuous action potential discharge volleys. 520
Applied to nerve, in nerve-muscle preparations: 0.01 - 0.1 ppm were active on neuraxon yielding rhythmic, spontaneous discharge at a potential lower than with DDT; 1 ppm gave rapid action on nerve trunks with spontaneous discharge in 1 minute; at more than 1 ppm caused blockage of nerve conduction, the effect being reversible by prolonged washing. 3278

(c) In Blatta orientalis:
Application to nerve cord yielded massive discharge followed by repetitive, synchronized, discharge of nerve impulses, then gradual decline to failure of all response. 204
(d) Claimed by some that pyrethrins act on peripheral nerve system of insects at a site only a few thousands of a mm from the external surface. 243

(9) "Pyrethrinization" and histopathology:
(a) Applied to Periplaneta as a concentrate via the 1st thoracic spiracle: I) Immediate initial paralysis; II) partial recovery in a few minutes; III) gradual decline with slower and slower peripheral movements; death. Movement of legs, heart and abdomen may continue more than 50 hours; $\frac{1}{2}$ - 52 hours after treatment no response followed electrical stimulation of the nerve cord. 260
(b) Tissue effects after the preceding treatment:
Analysis of nerve tissue in polarized light: Affected first the axoplasm then the lipid component of the nerve sheath where the most prominent lesions appeared. The birefringent ultrastructure was disorganized and lost prior to cessation of movement, but not prior to central nerve cord paralysis. The fine structure degeneration was progressive with time, extending from the region of maximum penetration and spreading. The action was selective on nerve tissue with degeneration of the axoplasmic colloids and of the nerve cell body plus later sheath degeneration and vacuolization. Normal post-mortem effects which followed death of the nerves from pyrethrum treatment complicated the histological analysis qua specific pyrethrum effect. 260
(c) Vacuolization of ganglia and nerve cord appeared in 10 - 20 minutes after the onset of convulsions in Corethra, the phenomenon being absent in larvae convulsed by sublethal doses. 1865
(d) In Melanoplus femur-rubrum and Tenebrio molitor (larva) lesions of brain, ganglia and connectives, were present 16 hours after external application of pyrethrins; vacuolization and disintegration of the involved nerve tissue was present and death was attributable to neuron destruction. Neuropathology is also described from Rhodnius, Cimex, etc. after pyrethrum treatments. 1420 1422 2000 3297

(10) Biochemical effects:
(a) In vitro preparations of Periplaneta americana coxal muscle cytochrome oxidase were completely inhibited (as measured by O_2 uptake in Warburg's apparatus) by pyrethrins and allethrin, at 10^{-5} M concentrations. 2305

(11) Metabolic:
(a) The reversible nature of pyrethrin paralysis suggests possible detoxification. Hydrolytic enzymes (such as esterases) attacking pyrethrins to produce non-toxic metabolites have been postulated. 6
(b) Prodenia eridania is reported to detoxify orally administered pyrethrins in 6 - 12 hours. 3348
(c) Incubated with blood, fat body, skin, muscle and intestine homogenates of Prodenia, pyrethrins were decomposed to various degrees, the fat body being most efficient in the breakdown. 3348
(d) Periplaneta - derived lipase hydrolyzed pyrethrins.
(e) Other metabolic mechanisms have been brought forward in Refs. 3336, 3397, 3333. 508

h) Temperature and the insecticidal action of pyrethrins:
(1) A negative temperature coefficient has been reported for pyrethrum in its action vs. Circulifer (= Euttettix) tenellus, Musca domestica, Blattella germanica, Macrodactylus subspinosus and Apis mellifera, with increased activity of the toxicant being noted at lower temperatures. 273
(2) Temperature and the action of pyrethrum vs. Periplaneta americana; C^{14} labelled (radioactive) pyrethrum in topical application: 273
(a) Topical LD_{50} (24 hrs) = ca. 1 μg/insect at 15°C or ca. 6 μg/insect at 35°C.
(b) At 35°C the rate of pyrethrum penetration into the interior of Periplaneta was more than twice that at 15°C.
(c) Insects, prostrate at 15°C from pyrethrum poisoning, may be returned to normal by transfer to an environment at 35°C; the process may be repeated over a period of several hours.
(d) Since pyrethrum treated Periplaneta, transferred from 35°C to 15°C become prostrate faster than those continuously held at 15°C, it is assumed that the insecticide (or some metabolic "toxin") was present in the vicinity of the action site at 35°C, but remained ineffective at that temperature.
(e) Pyrethrum poisoning in Periplaneta is stated to be associated with the presence of a haemolymph-borne "toxin" which does not show its toxicity at 35°C.
(f) Using C^{14} labelled pyrethrum it was shown that haemolymph from pyrethrum-treated roaches was not radioactive, indicating that a material in the blood and toxic to the insect is not pyrethrum.
(g) Bioassay of the blood of pyrethrum-treated Periplaneta using adult Sarcophaga crassipalpus indicated that symptoms of poisoning in the roach were correlated with the "toxin" content of the haemolymph.
(h) Piperonyl butoxide enhanced the susceptibility to pyrethrum of Periplaneta at higher temperatures.
(i) The "toxic principle" in the haemolymph of pyrethrum-treated Periplaneta lost its activity when stored at room temperature.

i) Synergism; pyrethrins and other compounds: (Also consult, Synergism General Treatment and individual synergistic compounds): 1755,353 2231,3037,2432
(1) Pyrethrum represents par excellence the insecticide for which synergistic action is both most clear-cut and well known.
(a) The temporary paralytic effect of small dosages of pyrethrins ("knockdown") and the lethal effects of larger doses are potentiated by from 2 to 12 times when pyrethrins are applied with certain synergistic substances, such as N-isobutyl undecylenamide, piperonyl butoxide, piperonyl cyclonene, n-propyl isome, ethylene glycol ether of pinene, N-(2-ethylhexyl)-bicyclo [2,2,1] -5 heptene-2, 3-dicarboximide, sesamin, etc., substances in themselves of very modest or no insecticidal power.

(b) The effect is one of true potentiation, the synergist (itself non-toxic or nearly so) permitting the use of quantities of pyrethrin much smaller than would be required to bring about a given toxic effect with pyrethrins alone. The synergistic effect abides when all considerations of droplet size, stabilization of insecticide and other physical effects are ruled out. 2432

(c) With sesamin and N-isobutyl undecylenamide the toxicity of pyrethrins is raised by a factor of 3 when up to equimolecular proportions of the stated substances are present; further increase of synergist brings in increased effect. Example: Vs. Aëdes aegypti the addition of synergist decreased the mean weight of pyrethrins needed to paralyze each insect from 6.0 to 2.0 x 10^{-7} mg. Once a 1:1 molecular ratio of pyrethrin + synergist was achieved no further fall in "knockdown" threshold quantity of pyrethrins was possible. There is postulated a complex (3 times as toxic as pyrethrins alone) acting at the peripheral nerve sheath interfaces and so reorientating the pyrethrin molecules that a more efficient discharge of resting potential at the interface occurs. 2432

(d) The sharp limitation of synergistic action when pyrethrin + synergist reach equimolecular proportions (noted with flying Aëdes) was confirmed for Sitophilus granarius crawling on deposits of pyrethrins (as oil films) from oil solution. Piperonyl butoxide seems exempt from the equimolecular limitation. 249

(e) Using pyrethrins and piperonyl butoxide or N-isobutyl undecylenamide as synergists vs. Musca domestica and Cimex lectularius the toxicity of pyrethrins was increasingly potentiated with a rise in ratio of synergist + pyrethins to at least 20:1; enhancement was greatest for the lower ratios for instance, falling off with further increase of ratio. 2344

(f) Piperonyl butoxide appears to be the most powerful synergist of pyrethrins, increasing pyrethrin potency vs. Musca by 5 times (allethrin by 4 times) and vs. Cimex by 2 times (allethrin by 3 times.) Isobutyl undecylenamide enhanced the potency of pyrethrins (+ allethrin) by no more than twice for either Musca or Cimex, using the measured drop or residual film methods. Piperonyl butoxide greatly prolonged the effectiveness of pyrethrin residual films. 2344

(g) Synergism (by independent joint action) is reported for pyrethrins and nicotine for Tribolium confusum in dipping tests. The maximum synergism observed was 2-fold. Application of nicotine, followed by pyrethrins at intervals of 0.75 to 6 hrs showed the greatest toxicity at the shortest interval; evidence of synergism became virtually nil with intervals of more than 6 hours. 3139

(h) A standard concentration of 2% piperonyl butoxide with 0.2% pyrethrins (formulated as water miscible emulsions completely free of mineral oils or objectionable ingredients) may be directly applied to grain in storage to control insects. Using 2-12 gallons of emulsion per 1000 bushels grain, protection was conferred for many months. 833

REPELLENCY OF PYRETHRINS FOR INSECTS:

1) Diataraxia oleracea (larva, final instar) is reported to be violently repelled on biting the leaf of a host plant treated with extract of natural pyrethrum. 3245

155

REPELLENTS

1) Action of certain repellent substances, deemed safe for man, on various mosquitoes; evaluated as average repellent time (time from application of the repellent to the recording of the first bite) in minutes. 3116

Repellent	Aëdes aegypti	Aëdes (sp) (Alaskan)	Aëdes taeniorhynchus	Anopheles quadrimaculatus	Average Repellent Time (Minutes) As Mixture* For A. aegypti	Aëdes sp. (Alaskan)
Acetoacetic acid, cyclohexyl ester	109	21	102	57	232	61
Bicyclo (2,2,1)-heptene-2,3-dicarboxylic acid, dimethyl ester, cis-dimethyl carbate	229	85	208	48	305	95
Cinnamic acid, propyl ester	173	-	237	74	263	32
1,2-Cyclohexane dicarboxylic acid, diethyl ester	176	-	96	57	344	-
Ethanol, 2-phenoxy-, acetate	166	46	100	53	261	49
Ethanol, 2,2′-thiodi-, diacetate	237	20	57	47	244	29
2-Ethyl-1,3-hexanediol	331	21	283	53	271	57
Hydracrylic acid, β-phenyl-, ethyl ester	262	6	83	42	169	15
Dimethyl phthalate	247	19	155	108	-	-
Phthalimide, N-sec.-butyl	201	11	77	55	274	62
Propionic acid, diester with 1,5-pentanediol	160	10	95	93	203	43
Indalone®	111	9	168	41	-	-
Succinamic acid, N,N'-dipropyl-, ethyl ester	322	101	182	61	203	68
Tartaric acid, diisopropyl ester	255	3	74	40	290	18

* Mixture: 6 parts dimethyl phthatate, 2 parts Indalone® and 2 parts of the compound listed in the first column of the table.

APPENDIX 2

BACKGROUND INFORMATION ON ZECTRAN

RESEARCH ON ZECTRAN [R]

A Substitute for DDT in the Control of Spruce and Jack Pine Budworm*

In 1964, an Insecticide Evaluation Project was established by the U.S. Forest Service at the Pacific Southwest Forest and Range Experiment Station in Berkeley, California. Its mission was to develop effective insecticides for major forest insect pests which would minimize environmental contamination. Two general approaches were to be followed: (1) find alternatives to conventional insecticides that were effective, nonpersistent, and specific or selective against the target insect; and (2) increase the efficiency of insecticidal treatments by improving formulations and application techniques.

A committee of Forest Service representatives agreed that the initial target insect would be the western spruce budworm, Choristoneura occidentalis. At the time, this insect was widespread throughout the Intermountain Region and parts of the Pacific Northwest. In 1964, for example the western spruce budworm caused damage to over 2 million acres of forest land in the Intermountain Region States alone. During the period 1949 to 1963, an average of 2,324,000 acres were sprayed in the United States and Canada for control of western spruce budworm.

Since about 1949, epidemics of the western spruce budworm were treated in this country by aerial spraying with DDT--usually a pound of insecticide per acre in a liquid formulation. With evidence mounting about the environmental hazards of DDT--mainly its persistence in the environment and its tendency to build up in food chains--the Forest Service was anxious to develop a safer method for controlling spruce budworm. Three conditions had to be met: (1) the insecticide should be more toxic to western spruce budworm than to other organisms; (2) the insecticide and its breakdown products must not accumulate in plants and animals in the forest ecosystem; and (3) the insecticide spray should be directed to the target insect with a high degree of efficiency.

Through research of the Insecticide Evaluation Project, a carbamate insecticide, Zectran, manufactured by Dow Chemical Co., has now been thoroughly tested and registered for use against spruce budworm, and two other insects--the eastern spruce budworm and the jack pine budworm. In initial laboratory tests conducted by the research group, Zectran was 20 to 25 times more toxic to the western spruce budworm than DDT. This meant it could be used in much smaller quantities with less risk of damage to other forest insects and wildlife. The parent compound and its metabolites broke down readily on exposure to air and sunlight, and in plant and animal systems. Although Zectran has a relatively high oral toxicity to mammals, it has a much lower dermal and chronic feeding toxicity--the main potential hazards in field use.

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*Revised from a July 1970 staff report of the Insecticide Evaluation Project, Pacific Southwest Forest & Range Experiment Station, Forest Service, USDA, P.O. Box 245, Berkeley, California 94701.

In extensive field tests conducted from 1964 to 1969, Zectran proved safe for handlers using it under field conditions. Considerable research has also been conducted on problem number three--the task of directing the spray with greater efficiency to the target insect.

One of the major problems in distributing a pesticide from the air is to get the spray to penetrate the forest canopy. Any vegetation acts as a filter, but coniferous forests are especially efficient in filtering out the larger drops of a conventional aerial spray which has droplets which may range from less than 1 to more than 350 microns in diameter.

Field tests conducted in 1965 and 1966 clearly indicated that only drops below 50 microns in diameter reached the western spruce budworm larvae with any degree of efficiency. Considerable effort from 1967 to 1969 was put into developing application techniques and equipment that would produce a higher proportion of small droplets and allow for most efficient use of the insecticide. The goal was to produce droplets less than 50 microns in size. Although progress in reducing droplet size was made by the Forest Service Equipment Development and Testing Center at Missoula, Montana, problems were still encountered in distributing the spray effectively within the forest canopy.

In 1969, because of the magnitude of the problem, the job of developing application techniques and special equipment for aerial spray operations was transferred to a new research unit at the Pacific Northwest Forest and Range Experiment Station at Corvallis, Oregon. Developing effective and efficient spray systems for applying Zectran or other compounds for forest insect control is now the responsibility of this research unit.

Zectran has been recommended for use against the spruce and jack pine budworm by the U.S. Forest Service. The insecticide and the system for its use has been registered by Dow Chemical Co. with the Pesticide Regulations Division of the Environmental Protection Agency.

This report summarizes the research on Zectran conducted by the Insecticide Evaluation Project in Berkeley since 1964, and documents the results of other research which have a bearing on the safety of Zectran for use in the forest environment.

PROPERTIES OF ZECTRAN

Zectran, chemically 4-dimethyl-amino-3,5-xylyl methyl-carbamate ($C_{12}H_{18}N_2O_2$), is a product of the Dow Chemical Company. In the past, it has been registered and sold for use against snails and insects on ornamental plants. Recently, it has been taken off the market by Dow because of the demand for its use in research programs.

Zectran belongs to a group of insecticides known as carbamates which in general are safer than many other common insecticides. Animals exposed to carbamates have a very rapid recovery from cholinesterase inhibition. Cholinesterase activity is necessary to the normal transmission of impulses in the animal nervous systems. Insecticides affect the transmission of nerve impulses in such a way that the transmission is continuous. In Zectran, this effect is of very short duration and is rapidly reversed (1). Recovery from carbamate induced cholinesterase inhibition is quite rapid, usually within a half hour. This indicates that Zectran would not produce a cumulative cholinesterase inhibition as is the case with some organophosphorous insecticides.

Zectran also has some desirable properties which distinguish it from many other carbamates. It is selective against the western spruce budworm, being more toxic to the budworm than to most other forest insects, and it does not persist in the environment.

LABORATORY TESTS

Toxicity of Zectran

To Western Spruce Budworm

The toxicity of Zectran to western spruce budworm larvae has been determined by applying the insecticide topically (on the surface) to the 6th instar, a stage in larval development. Zectran proved unusually toxic to the budworm--in fact, 20-25 times more toxic than DDT. Zectran has shown the same level of activity against western spruce budworm as pyrethrins, a compound which has almost invariably been the most toxic candidate in tests on 21 species of lepidopterous insects (2,3,4).Both sexes of western spruce budworm are equally affected. Zectran was considered more promising than pyrethrins because pyrethrins are more costly and because they break down so rapidly in sunlight that they would be nontoxic before reaching the insects. Since this work was done, however, the Insecticide Project has succeeded in developing a stabilized pyrethrin formulation which also has promise for insect control.

The toxicity of Zectran as a spray was determined in a laboratory spray chamber using 6th instar larvae. The dosage needed to produce 90 percent kill in the spray chamber was:

	Ounces per acre
Zectran	0.66
Pyrethrins	.54
DDT	14.00

Populations of western spruce budworm from both Montana and New Mexico were tested and found equally susceptible to Zectran sprays in the laboratory.

Tests of sprays against larvae in various stages of development showed Zectran to be slightly more toxic to the 2nd, 3rd, and 4th instars than to the 5th and 6th instars. The toxicity of Zectran to the life stages in descending order was: Adults, larvae, (6th instar), pupae.

Oral toxicity of Zectran to the western spruce budworm was higher than pyrethrins by 1.7 to 2.6 times and was 2.3 to 5.3 times higher than DDT.

To Other Organisms

The acute oral, chronic feeding, and dermal toxicity of Zectran has been thoroughly investigated by Dow Chemical Co.; the U.S. Department of Interior at its Patuxent and Denver Laboratories; the U.S.D.A. Forest Service; the Department of Entomology of the University of California at Berkeley; and by Russian scientists (14).

The acute oral toxicity of Zectran for rats ranges from 13-65 milligrams per kilogram of body weight (mg./kg.). This means Zectran has a relatively high oral toxicity for mammals. Dermal toxicity, however, is extremely low. Normal handling precautions are sufficient to safeguard those working with it. Dermal toxicity was nil at 2,000 mg./kg. for rabbits.

Studies by the U.S. Fish and Wildlife Service at Denver indicate that Zectran has no cumulative effects. For example, daily doses of Zectran producing symptoms of toxicity could be tolerated by mule deer for months without any permanent determental effects (5).

Birds are very susceptible to the effects of many insecticides since they have very high metabolic rates. But birds have shown a high tolerance to daily doses of Zectran. While oral doses of 3.0 to 5.2 mg./kg. of Zectran killed 50 percent of a test population of mallards and chukar partridge, these birds could tolerate 40 percent of an LD/50 for 30 days. (LD/50 refers to that amount of insecticide required to kill 50 percent of the test population.) Reproduction of the treated chukars was similar to that of the controls (5).

Zectran is especially safe to fish. It is one of the least toxic chemicals tested on game fish by the U.S. Fish and Wildlife Service at their Fish Pesticide Laboratory in Denver. For example, the toxicity of Zectran to fish is much lower than that of DDT. Toxicity with fish is measured as lethal concentration required to produce 50 percent mortality in a test population of fish (LC/50). For Zectran, the LC/50 is 5.3 milligrams per liter of water. For DDT, it is 0.002 mg./l. (6).

Zectran has also been tested on bullfrogs, which exhibit a high tolerance to the insecticide. LD/50 ranges from 283 to 800 mg./kg. (5).

The hazard to aquatic insects is also quite low. The LC/50 is below 0.1 mg./l.

Persistence of Zectran

Zectran has been shown to be a nonpersistent insecticide, breaking down in sunlight and air within a few hours. In laboratory studies, the residual activity of Zectran and DDT was compared by spraying potted Douglas-fir trees with doses which were equivalent to 0.5 oz./acre of Zectran and 14 oz./acre of DDT. The deposits were allowed to age outdoors before caging insects on the treated trees. The residual life of Zectran was very short showing almost no toxicity to budworm after two days. The toxicity of DDT was essentially unchanged after the same length of time (table 1).

Studies in the field have shown that Zectran and its breakdown products do not accumulate or persist in the environment. Residues of Zectran on Douglas-fir and five common browse plants were determined after the 1966 field tests in Montana. Zectran levels dropped rapidly in most of the plants investigated after the first day (table 2). The amount remaining after one week was negligible. All Zectran residues were insignificant within a month (7).

Breakdown Products

Like most insecticides which are nonpersistent, Zectran breaks down into other compounds, both in sunlight and air and in plants and animal systems. These breakdown products have been studied, both in the field and in the laboratory, to determine if they are toxic or persistent.

Photo-oxidation plays a strong role in the breakdown of Zectran (8). Three hours exposure to ultraviolet light led to the production of 6 or more compounds that were shown to be cholinesterase inhibiting. More intensive breakdown would probably occur with additional exposure. This is backed up by the work of Abdel-Wahab *et al* (9), who have found that Zectran completely disappears from the surface of bean plants in two days, degrading into 8 or more breakdown products. The rapid degredatio of Zectran is accompanied by a corresponding disappearance of its metabolites.

The major metabolites were found to be methylamino, amino, methyl formamido, and formamido Zectran. Three of these metabolites showed cholinestase inhibition. Both methylamino and amino showed toxicity to rats in the same range as Zectran. These compounds in turn are rapidly degraded to non-toxic materials.

Toxicity of the four major breakdown products of Zectran to western spruce budworm has been determined by topical application. All were substantially less toxic than Zectran.

The breakdown of Zectran has also been studied in some other insects (10), plants and animals. Working with larval insects, Roberts found that Zectran was 50 percent metabolized within 3 hours after topical application to the spruce budworm, tobacco budworm (*Helio virescens*), and housefly (*Musca domestica*).

Table 1--Residual life of Zectran and DDT on potted Douglas-fir trees. Bioassay with 6th instar western spruce budworm[1]

Aging period outdoors	Corrected mortality (7-day post-treatment count)	
	Zectran 0.5 oz./acre	DDT 14 oz./acre
hours	--- percent of insects killed ---	
0	90	98
4	66	--
24	36	90
48	7	95

[1]Insects caged on foliage after deposit was exposed to sunlight outdoors.

Table 2--Zectran found after period indicated (PPM by species)

Species	Days								Weeks			Re-covery factor
	0	1	2	3	4	5	6	8	2	3	4	
												percent
Pseudotsuga menziesii	2.86	0.19	0.22	0.15	0.14	0.17	0.14	0.17	--	0.13	--	77
Balsamorhiza sp.	7.85	.47	.33	.28	.94	.82	.94	.13	0.22	.04	0.00	85
Ceanothus sp.	1.29	1.56	.94	1.25	1.52	1.56	1.00	.67	2.01	--	.19	48
Fragaria sp.	6.25	4.17	4.58	2.19	5.73	2.71	3.10	--	.73	.94	.38	48
Taraxacum sp.	2.29	.44	.11	.11	.08	.04	.01	.13	.07	--	0.00	70
Tragopogon sp.	.75	.56	.29	.06	.04	--	.04	.01	.01	--	.11	80

Mode of Action of Zectran

Insects

Considerable research has been conducted in an effort to determine when, where, and how Zectran is degraded in the insect. This research may, in the future, lead to design of even safer insecticides. Zectran is 50 percent metabolized, or broken down, within 3 hours after topical application to the western spruce budworm, tobacco budworm, and housefly (10). Because research has shown that insecticides are detoxified at certain sites in insects, scientists felt that this might also be the case with Zectran and the western spruce budworm. An attempt was made to produce metabolism of Zectran in tissues and subcellular fractions isolated from the western spruce budworm and housefly larvae.

Almost every tissue and subcellular fraction of the spruce budworm larvae has been tested. With only one exception, scientists have not been able to produce the same rate and type of oxidative metabolism of Zectran found after topical application to live larvae. Researchers have tested most of the *in vitro* systems mentioned in the literature, including adding the oxidative enzymes tyrosinase or polyphenol oxidase into the insect homogenates. There was still no metabolism of Zectran.

The metabolism oxidation of Zectran may occur only in or on the cuticle of the western spruce budworm larvae. This oxidation may be independent of enzyme activity, but perhaps aided by some organic or inorganic constituent on the surface of the cuticle. Because it is especially important to determine exactly how the insecticide is broken down by the insect, research on this is continuing.

Plants

Considerable effort has gone into the study of the mode of action of Zectran in plants, especially in connection with research aimed at producing systemic insecticides.

Penetration studies have been conducted with cuticular membranes isolated from white fir, Douglas-fir, ponderosa pine, apricot, agave leaves, and tomato fruit. Zectran is one of over 30 compounds which have been evaluated.

Fat-soluble carbamates such as Landrin penetrated readily while Zectran penetrated more readily than did the following: halogenated hydrocarbons, pyrethrins, organophosphates, triazines, pyridillium chlorides, substituted ureas, sucrose, and glucose. The thickness of the cuticle did not correlate with penetration.

The movement of Zectran across or through isolated plant cells has also been studied in the laboratory. The data show that Zectran is absorbed in living cells by a diffusion process and is not actively pumped into the cell cytoplasm at concentrations greater than those in the cell wall or surrounding solution. Zectran diffuses across cell membranes more effectively than all the organophosphorous insecticides studied. However, insecticides such as SD-8530 and DDT diffuse more rapidly because they are more fat soluble.

Physiological Effects of Zectran

As indicated elsewhere in this report, the oral toxicity of Zectran to mammals is quite high. The dermal toxicity of Zectran to mammals, however, is very low, and normal handling precautions would be sufficient to safeguard those working with it.

If Zectran is applied in the manner recommended in this report, the probability of its reaching human beings in quantities large enough to produce damage of any kind is extremely low. However, no discussion of the safety of an insecticide would be complete without an analysis of its potential for producing cancers or birth defects. The authors of this report have conducted no research in this area. The following conclusions have been drawn from the available literature.

Dow Chemical Company had carcinogenecity studies of Zectran carried out by the Wisonsin Alumni Research Foundation. Their report did not indicate any cause for concern.

The Mrak Commission has classified Zectran in a group of chemicals that should not be used on food crops until further evaluation for effect is carrried out.

During the period 1965-68, the Bionetics Research Laboratories of Litton Industries, under contract to the National Cancer Institute, tested various pesticides and related compounds for possible teratogenic effects (birth defects). Zectran is one of a number of compounds tested by injecting the insecticide beneath the skin of mice. When applied at a rate of 10 mg./kg. of body weight in a DMSO solvent, Zectran produced no significant increase of anomalies in the two strains of mice tested (11).

FIELD TESTS

The first small-scale field test of Zectran against western spruce budworm was conducted on the Salmon National Forest in Idaho during the summer of 1964. Three 20-acre plots were sprayed at the rate of 0.1 lb./gal. of Zectran per acre, resulting in an 88 to 92 percent reduction of the budworm population.

In 1965, Zectran was tested again in the West Fork Ranger District of the Bitterroot National Forest of Montana. This resulted in a 91 percent reduction of the insect population.

In conjunction with the 1965 test, an additional 335 acres were sprayed with the same Zectran formulation but with fluorescent particles added. This was done in an attempt to measure the drop size reaching the insects and to determine how well the insecticide was being distributed. This test resulted in a population reduction of 98 percent.

In early spring of the 1966 test, a test was conducted in the drainage of the West Fork of the Bitterroot River, also in the Bitterroot National Forest. There were several objectives: (1) to test

Zectran at 0.15 lb./gal. per acre against western spruce budworm under normal spraying conditions in the field; and (2) to allow scientists to try new methods for determining spray distribution and budworm mortality; and (3) to monitor the effects of Zectran on several forms of wildlife, including small mammals, song birds, grouse, fish, and other aquatic organisms. Two spray areas were selected, one 3,538 acres in size, the other 1,190 acres. Two check areas of 1,000 acres each were also established. Spraying was accomplished with a fixed-wing aircraft. The formulation consisted of one part Zectran/Dowanol mixture diluted with nine parts deodorized kerosene. This produced the desired dosage of 0.15 lb./gal./acre of Zectran. Fluorescent particles were also used in this test.

The 1966 test was evaluated by two separate sampling plans. One, using 50 one-tree plots throughout both sprayed areas, indicated an 87 percent population reduction in the 3,538-acre area and a 77 percent population reduction in the 1,190-acre area. A corresponding 44 and 49 percent population reduction occurred in the check areas. A second evaluation was made with the cluster-sampling method (used in previous years) in the 3,538-acre area. In this case, a 94 percent population reduction was indicated (12).

In 1967, two spray tests were made. One was in the Sawtooth National Forest, Idaho, the other in northern Maine. Due to a catastrophic decline in the spruce budworm population during the spring in the Sawtooth area no reliable data were obtained on population reduction. However, the test in Maine gave valid results. The formulations consisted of 75 pounds of Zectran dissolved in 50 gallons of Dowanol TPM. This mixture was applied as a fine spray at the rate of 13 fluid ounces per acre, still 0.15 pounds of Zectran per acre. Approximately 500 acres were treated resulting in an 82 percent budworm reduction.

The foregoing outlines the effectiveness of Zectran applied at 0.15 lb./acre. Subsequent tests have been conducted varying the dose and method of application to try to further reduce the dose of insecticide.

During 1968, a test was conducted against the western spruce budworm in Belmont and Chamberlain Creeks in the Blackfoot River drainage east of Missoula, Montana. Zectran was applied at a rate of 0.06 lb./acre in 0.125 gal. Dowanol TPM with a droplet size no larger than 120 microns. Spruce budworm mortality was 70 percent in the Belmont Creek area, but very low in the Chamberlain Creek area. The reasons for this are not completely understood. When using ultra-low-volume application or with very tiny spray droplets, air currents play a major role in distribution of the insecticide. A better understanding of the meteorological condition in mountainous regions will be essential in order to take full advantage of atmospheric transport and diffusion in delivering the spray to the insec

In 1969, another test of Zectran was carried out in the Nezperce National Forest in Idaho. Two areas were selected, each about 4,000 acres. One was sprayed at a rate of 0.15 pounds of Zectran in 1/2-gallon of TPM. The other area was sprayed twice with 0.075 pounds of Zectran in 1/2-gallon of TPM. Results indicated less than 80 percent mortality in both areas. There was no significant difference between the plot sprayed once and the area which had been sprayed twice.

ENVIRONMENTAL EVALUATION

Only a small fraction of the pesticides used in the United States are applied to forest lands. Yet forests are an especially important part of the total environment. They are the sources of major water supplies and home for most of our wildlife. The application of pesticides to forest lands must be done with great care, and with as little effect as possible on other insects, fish, birds, and other wildlife.

In the course of field testing Zectran for operational use against the western spruce budworm, scientists have also investigated its potential effect on the rest of the forest community. Intensive studies of the effects of Zectran on non-target insects, fish and wildlife, and its residual characteristics in soils and plant tissues have also been made.

Terrestrial Insects

Results of the 1965 and 1968 field tests showed that Zectran is more toxic to western spruce budworm than other forest insects. Zectran reduced western spruce budworm populations to a much greater extent than it reduced populations of most insects found associated with it in the different crown levels (12).

Results of the 1965, 1966, 1968, and 1969 field tests showed that parasitism of western spruce budworm by Apanteles fumiferanae and Glypta fumiferanae increased following treatment with Zectran (13).

Drop cloths were set up during the 1965 spray test in Tough Creek and Mud Creek to catch dead insects. Results of these tests support the results obtained by sampling defoliator populations in the tree crown. Zectran was more effective against defoliators than was naled, another insecticide being tested.

Insect traps designed to catch flying terrestrial insects were set up in the Trapper Creek and Violet Creek Areas in 1966 and 1967, and in Tough Creek (1965 field test) and One-two Creek in 1967. Terrestrial insect populations in Tough Creek appeared to be fully recovered from any effects of Zectran by 1967. The number and variety of insects were similar those of unsprayed check areas.

Aquatic Insects and Fish

The effects of Zectran on fish and wildlife have been studied intensively both in the laboratory and in the field. During the 1965 Zectran test, the Bureau of Sport Fisheries and Wildlife undertook a study of the effects of Zectran on trout and aquatic organisms. No direct effect of Zectran were observed on live-caged trout. Fish reacted normally throughout the test. There was an increased drift of dead insects in the stream at all sampling stations, including the check stations, immediately after spraying.

In a study conducted in 1966 on the Salmon National Forest, there was no significant increase of emigration and intrastream movement of fish. No effects on benthic aquatic insect numbers were observed; however, more insects were observed drifting downstream about three hours after spraying. This continued for several hours. Adult terrestrial insects and immature aquatics (Heptageneidae, and Phyacopheledae, and Blepheracendae) increased in drift samples after spraying. The conclusion is that Zectran disrupts aquatic insect populations less than DDT, malathion and diasinon.

Further studies of the effect of Zectran on aquatic insects were conducted in Maine during the 1967 tests. No significant change in insect numbers was evident in either bottom samples or drift samples following spray. Zectran had no observed effects on live-caged Eastern brook trout. No dead fish were observed in blocking nets. These results agree with those obtained in the 1967 tests in Idaho.

Birds and Mammals

Extensive studies of the effects of Zectran on wildlife were conducted by the Bureau of Sport Fisheries and Wildlife, U.S. Department of Interior. In a 3-year field study beginning in 1965, in the Bitterroot National Forest, their work showed that Zectran had no discernable effect on birds or small mammals. In general, their observations indicated that: (1) no harm to birds or mammals resulted from the Zectran applications; (2) the spray temporarily increased the availability of budworm larvae and other insect food for birds; and (3) that the reduction in available food that followed this increase was not sufficient to cause birds to abandon their nests or enough to interfere with rearing of the young. The Bureau of Sport Fisheries and Wildlife concluded that over a period of three years, Zectran . . ."has shown no important detrimental effects to wildlife." (14).

The Montana Fish and Game Department investigated the effects of Zectran on grouse for a 2-year period in an area sprayed in 1966. They also set up a special plot that was purposefully sprayed with 5 times the usual dose of 0.15 pound of Zectran per acre. With the use of banding, color marking, and radio telemetry, wildlife biologists were able to study subsequent movement of the birds. A summary of their results (15) follows.

The strongest evidence that Zectran did not affect blue grouse survival or behavior was obtained from the multiple-sprayed Mud Creek Unit. Seven of 11 banded adult males, positively exposed to Zectran, survived to the following breeding season (10 months post-spray). An eighth banded male exposed to Zectran survived until fall (4 months post-spray), when taken by a hunter.

Radio-telemetry showed that survival and behavior of seven brood hens in the heavily sprayed area was normal for 37 days following spraying. Repeated post-spray flush counts of the broods with instrumented hens gave no indication of chick mortality in six of seven broods. Chick loss occurred in one brood shortly after spraying; whether this was a direct effect of the pesticide or natural mortality is not known.

Some observations were also made from the Trapper Creek test (1966) where Zectran was applied at 0.15 lb./acre. The area was sprayed between June 30 and July 4. Only nine of the 45 blue grouse males on Trapper Creek study units are known to have been exposed to Zectran. Five of 21 females banded on Trapper Creek units were observed after spraying. Three of the five females were radio-equipped and monitored within the sprayed area for periods of from 7 to 19 days after application. The Trapper Creek study provided the only field evaluation for the effect of Zectran on ruffed grouse. Four of five banded males were observed to have survived for varying periods after the spray. There were no significant differences in the annual survival rates of banded blue grouse males on sprayed and unsprayed study areas. In the same period, only 15 of the more than 75 blue grouse which had been banded in the same areas were located and identified. The only ruffed grouse located in the post-spray observation period were instrumented with transmitters.

None of the instrumented grouse died or showed signs of poisoning. The movement of instrumented grouse in relation to the spray patterns was determined. Eleven of the 13 instrumented grouse exposed to Zectran remained in sprayed zones for at least a week after spraying--the period when the relatively short-lived Zectran would be potentially the most hazardous. Limited telemetry studies did not reveal changes in respiration or activity pattern of instrumented grouse after they were exposed to aerially-sprayed Zectran.

Soils

Soil studies (16) indicate that Zectran has no effect on microbial respiration when applied to soil as a dry powder, combined with soil litter, or applied in an acetone-oil carrier. Even when Zectran was applied to soil in concentrations far greater than 0.15 lb./acre, it had no significant effect on microbial activity. When properly applied, Zectran should pose no hazard to microbes in the soil.

RECOMMENDATIONS FOR USE

Zectran is recommended for control of western and eastern spruce budworm and the jack pine budworm by means of an aerial application of 0.15 pound technical Zectran per gallon per acre. Formulation consists of a glycol ether concentrate of Zectran diluted with 9 volumes of deodorized kerosene.

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APPENDIX 3

Background Document on Bt

I. General Information

A. Common Name

This pesticide is commonly referred to as Bt. The name derives from the initials of the active ingredient Bacillus thuringiensis, an aerobic spore-forming, crystal producing member of the bacterial genus Bacillus.

B. Chemical Name

There is no chemical name for this pesticide since it is based on a living bacterium.

C. Registered Uses

In terms of forest insect pest control, Bt products are registered for use against the following insects (ground application only)

a. Spring and fall cankerworm.

b. Great Basin tent caterpillar.

c. Fall webworm.

d. California oakmoth.

e. Red-humped caterpillar.

f. Gypsy moth.

g. Elm spanworm.

D. Formulations Manufactured

Bt is available in three basic formulations, i.e.

a. wettable powder

b. aqueous concentrate

c. dust

E. Dilution of formulations for use.

Water is the generally accepted diluent for wettable powder and aqueous concentrate formulations. Glycol and oils have been used experimentally, but were generally not as good as water for a diluent. Dust formulations are not diluted.

F. Rates and Method of Application.

At present, the only registered method of application is by ground equipment for forest pests (hydraulic and mist blower).

Rates of application of the active ingredient, depending upon the insect species, range from 0.25 - 4 pounds of wettable powder and 0.50 - 2 quarts of aqueous concentrate per acre.

G. Tolerances in food.

All Bt products are exempt from tolerance limits. (See Federal Register Vol. 36(228) section 180.1011.)

H. Manufacturer

a. Bactospeine* - French manufactured and registered. Not registered in U.S. Distributed in U.S. by Rhodia, Inc. Chipman Div.

*Trademark of Pasteur Institute, used by Laboratory Roger Bellon.

b. Biotrol XK - EPA Temporary permit No. 6296-EXP-4G. Manufactured by Nutrilite Products, Inc., Lakeville, Calif. 92353. Distributed by Thompson-Hayward Chem. Co., Kansas City, Kansas 66110.

c. Dipel - EPA Registration No. 275-18. Manufactured and distributed by Agricultural and Veterinary Products Division, Abbott Laboratories, North Chicago, Ill. 60064.

d. Thuricide-HPC - EPA Registration No. 4456-541. Manufactured and distributed (bulk) by International Minerals and Chemical Corp., Libertyville, Ill.

II. Toxicity data on formulations

A. Safety data

1. Acute Mammalian Studies

a. Thuricide was administered to rate in a 33% suspension in water with carboxymethyl cellulose as a thickener. The dose was placed in the stomach by means of a ball-tipped hypodermic syringe. Doses up to 24 grams of Thuricide ($2x10^{12}$ spores) kilogram were administered to groups of 10 rats. The animals were observed for 1 week. No fatalities occurred. No outward symptoms of toxicity. Gross and histological

examination of tissues showed no differences from tissues of control animals. (1,2)

Eighteen humans ingested 1 gram of Thuricide daily for five days. Complete physical and laboratory examinations were given prior to the experiment, at the end of the five day ingestion period, and 4-5 weeks later. Physical examinations included detailed history and records of height, weight, temperature, blood pressure, respiratory rate, pulse rate immediately after exercise and 30 and 60 second thereafter. Evaluations were made of genitourinary, gastrointestinal, cardiorespiratory, and nervous systems. Lab tests included routine urinalysis with qualitative and quantitative urobilinogen determinations (when indicated), complete blood count, sedimentation rate, blood urea N, glucose, bilirubin and thymol turbidity tests. All subject remained well during the course of the experiment. All laboratory findings were negative. (1)

Dipel was fed to groups of 10 ♂ mice (16 - 25 gms) at the rate of 10 gms/kilogram (1.5×10^7 I.U.). No mortality occurred. LD_{50} was beyond 10 gms/Kg. (11)

Dipel was fed to 3 female mongrel dogs. Dosage was 400 mg/kg (6×10^6 I.U./Kg). The animals were sympton free during the 48 hour observation period. (11)

b. Dermal

Dermal effects of Bt were tested by application to shaved flanks and bellies of albino rabbits. Dosages used ranged from 20% suspensions to 50 mg/ animal. After application half of the treated skin was abraded while the other half was left intact. Readings were made at 24, 48, and 72 hours in one test and up to three weeks in another. Other than local, mild erythema, no ill effects were noted in any test animal. (1,2)

In another study dermal application to albino rabbits was made to test allergenicity response. Ten sensitizing doses were applied every other day for three weeks. Readings were made 24 hours after each application of Bt. Two weeks after the tenth application a challenge dose was applied. Only slight erythema and edema were noted. No allergenic response was elicited. (1)

Allergenicity was also tested with guinea pigs following the procedure of Draize. No allergenic response was noted. (1)

c. Inhalation

Bt inhalation studies were conducted on mice, rats, guinea pigs, and human volunteers.

In one test with mice, the animals were exposed to 10 gms of Bt powder for 15 minutes. Dosages were applied four times over a period of six days. No ill effects were noted and gross pathology was negative. (1)

In other tests with rats and guinea pigs, exposure to a 10% Bt preparation for 10 minutes, no fatalities were recorded for the one week observation time. Dyspnea (discomfort) was noted but recovery was rapid. The animals showed normal weight gain. (1)

Five human volunteers inhaled 100 mg Bt powder daily for five days. Complete physical examinations prior to the test, immediately after the test, and 4-5 weeks later showed no abnormal conditions in the test subjects. (1)

d. Eye irritation

Ocular irritation with Bt was tested in albino rabbits. 0.1 cc of a 20% suspension was instilled in each eye. One eye was immediately rinsed with isotonic saline. Six animals were tested. The eyes were examined immediately, after 3 hours and 24 hours, and every 24 hours until they appeared normal. Slight redness of the eyelids was noted at 3 hours and 24 hours. Eye irritation disappeared in 48 hours. (1)

2. Subacute and chronic studies

Bt daily administered as the spore-crystal complex to rats for 3 months at per os rates of 25, 100, and 400 mg/kg produced no main function disorders or organ damage. Similar results were obtained in dogs fed 6, 25, and 100 mg/kg for three months. (1,2)

3. Other studies

Birds

Avian and fish toxicity tests have been conducted with several Bt preparations.

One long term study with 6 New Hampshire Red laying hens was conducted over a 23 month period. The hens received a daily dose of Bt ranging from 0.5 - 10 gms per bird. Results showed no allergic response, other illnesses, or variations in the expected egg production of the hens on test diet. There was no significant difference between the test birds and the birds used as controls.

In a 9 week oral toxicity test administered to 24 groups of 10 chicks each, no significant differences of any kind were noted between the test and control groups of chicks. (12)

Seventeen pheasants and 2 partridges, all about 6 weeks old, were divided into two groups. One group

was fed 1.0 gm of Bt/bird/day in two gelatin capsules. The control groups were fed two empty gelatin capsules daily. (4)

No deaths or symptoms of respiratory, alimentary or other disturbances were noted in the Bt fed group. Two pheasants in the control group died of trauma (due to handling). Birds in both groups exhibited feather color and pattern, bearing and weight gain that is expected in similar groups of birds in nature.

It was concluded that no differences in behavior or development of test birds resulted from the ingestion of 1.0 gm of Bt spore-crystal complex per bird per day when compared to the control group.

In an oral acute toxicity study conducted with Bt in young adult bobwhite quail showed the acute oral medial lethal dose to be in excess of 10 gm/kg body weight. Five male and five female quail were fed 10 gm/kg by gavage. A similar group was fed distilled water as control. (12,13)

The test period was 21 days. At the end of the test period all animals were sacrificed and subjected to a gross pathological examination. No pathology attributable to the test material was found. Growth rate was similar in the test and control groups.

Fish

Several acute fish toxicity tests were conducted with Bt preparations.

A four day fish toxicity study was conducted with Bt employing rainbow trout and bluegills as test animals. Two groups of ten fish each were placed in water containing Bt at concentrations of 560 and 1000 ppm. As a check each lot of experimental fish was challenged with a reference pesticide, pp^1-DDT. None of the trout or bluegills exposed 96 hours to Bt at concentrations of 560 and 1000 ppm died. The 96 median tolerance limits (TL_{50}) of pp^1-DDT for the same lots of fish were calculated to be 0.024 ppm for trout and 0.032 ppm for bluegills. (1)

In another test with rainbow trout, results showed no acute effects on 2.5" trout exposed to 1-10 mg Bt/Kg water. (1)

Larger rainbow trout - 4" in length were exposed to Bt at concentrations of 100-1000 ppm for 14 days. No deaths or symptoms of alimentary or behavioral disturbances were evident. (1)

In a test with juvenile Coho salmon (1.6" in length), Bt was about 1/30 as toxic as DDT. The tests ran for 168 hours with concentrations of 8-406 mg Bt/1. water. (1)

The 48 hour median tolerance limit of the Bt was about 50 mg/l. whereas that for DDT was 0.08 mg/l water.

Effects on beneficial insects

A. Bees

Numerous tests with several serotypes of Bt showed no deleterious effects where practical dosages were used. Heat stable exotoxin is poisonous to bees as is high concentrations of spores. (15)

B. Silkworm

This domesticated insect is highly susceptible to Bt particularly var. thuringiensis. Because of this, it is illegal to disseminate foreign preparations of any pathogen in Japan. (15)

B. Physical - Chemical Properties

Inasmuch as all commercially available Bt products are biological materials they do not have all the physico-chemical properties of chemical insecticides.

Bactospeine, Biotrol, and Dipel are marketed as wettable powders or dusts and do not have boiling points, flash points or vapor pressure. All three materials are stable for more than a year at commercial warehouse or laboratory conditions. These wettable powders are

insoluble in normally used carriers such as water, spray oils, etc. However the primary active ingredient, the crystal, will solubilize under high pH conditions (9.5 plus).

Thuricide HPC, the fourth commercial product available in the U.S., is marketed as an aqueous suspension. It must be protected from freezing and high temperatures (above 90°F). The product is stable for several months. Thuricide has no boiling point or flash point. Its' density is 1.07 at 90°C. The material steams off without boiling and the solid material remaining decomposes without melting.

III. Efficacy data under field and laboratory conditions

Most of the laboratory and field efficacy data were obtained on preparations of Bt based serotype I strains. U.S. produced Bt preparations are no longer based on serotype I strains. A brief resume of field efficacy of these earlier tests against forest insects is given below.

Gypsy moth

Reported field trials in the period 1961-66, indicated improved results in attempts to control the gypsy moth with Bt. These improved results were primarily due to improvements in the commercially available formulations. Evaluations were based primarily on population reduction (egg mass reduction or direct larval mortality) or on foliage protection. The consensus of these reports indicated variable results,

particularly from aerial treatment. Some foliage protection was noted, but populations of the insect were not reduced to the levels desired. (8,9)

Spruce budworm

1961-65 field tests with Bt in Canada and the U.S. indicated that unacceptable population control could be achieved by Bt. Evaluations were based primarily on population reduction (larval and pupal mortality and residual egg masses). (16)

Linden looper

A 1962 field test using Bt products resulted in 100 percent control of the linden looper.

Great Basin tent caterpillar

A large scale field test with Bt against this tent caterpillar gave acceptable population reduction results. Populations were 95% lower in treated areas as compared to untreated areas one year after application.

California oak moth

Field tests with Bt against this insect gave acceptable population reduction results. Complete control of the insect was reported.

Laboratory tests

Virtually all major forest insect lepidopterous pests proved to be susceptible to the killing effect of Bt. However

many of the field tests resulting from favorable laboratory tests failed or gave unacceptable control effects. The reasons for these field failures are usually ascribed to:

1. Lack of persistence in the field.
2. Poor application techniques.
3. Effects of environmental factors such as U.V., rain, foliage effects.
4. Poor formulations.
5. Instability of product.
6. Inadequate coverage particularly from the air.
7. Insufficient field effects of the Bt strain used.

In 1970, Bt for the control of insects took a new turn. A new isolate HD-1, identified as a variety Kurstaki serotype III, was propagated and exhibited a much greater degree of effectiveness than earlier preparations.

Laboratory tests showed that the new isolate was 10-100 times more effective than commercial preparations based on the serotype I, berliner strain.

This new isolate was put into commercial production and now forms the basis for the three commercially available U.S. products.

The first laboratory and field efficacy trials with these new products were conducted against agricultural crop pests, such as the cabbage looper. Crop damage evaluations

and population reductions compared favorably with chemical insecticide controls.

Laboratory evaluations of these new products gave encouraging data, so that several field tests were conducted in 1971 against selected forest insect pests, such as the gypsy moth, spruce budworm, elm spanworm, saddled prominent, and hemlock looper. Although the results of these field trials has not appeared in print, a summary of some of the results can be made.

Gypsy Moth

Field efficacy tests were conducted in several areas in the Northeast. Ground mist blower tests using Bt at 8 and 16 billion International Units per acre gave excellent foliage protection in all cases and some degree of population control as measured by egg mass changes (personal communication, Metterhouse, W., Becker, W., Yendol, W., and Dubois, N.). Applications were made three times at 10 day intervals.

An aerial application of the new Bt was reported as highly successful in population reduction and foliage protection (personal communication, Secrest, J.). Two applications of 8 billion International Unit per acre in one gallon of water were used. A molasses based adjuvant was used in this new formulation.

and population reductions compared favorably with [illegible] insecticide controls.

[illegible] Laboratory evaluations of these [illegible] encouraging data, so that several [illegible] in 1971 against selected forest [illegible] gypsy moth, spruce budworm, elm spanworm, and hemlock looper. Although the results [illegible] obtain has not proceeded to print, a summary [illegible] results can be made.

In the [illegible]. Ground mist blowe[illegible] and 16 billion International Units [illegible] foliage protection in all cases and some [illegible] control as measured by egg mass changes [illegible] Doane, Wallis, Hetterhouse, W., Becker, [illegible], Yendol, W., [illegible] applications were made [illegible] times at [illegible]

An aerial application of the new [illegible] highly successful in population reduction [illegible] protection (personal communication, Dr. [illegible]) [illegible] applications of 8 billion International Units [illegible] of [illegible] were used. A-woolsorb used adjuvant [illegible]

Ground application of the new Bt products in water only gave excellent control of the elm spanworm (personal communication, Kaya, H.).

Foliage protection was achieved against the gypsy moth, but population reductions were not acceptable.

Spruce budworm

Aerial application of the new Bt in an experimental formulation gave excellent foliage protection and population reduction when applied twice against the spruce budworm in Canada (personal communication, Smirnoff, W.).

The formulation tested in Canada was glycol based and was applied by spinning disc nozzles.

It should be pointed out that field efficacy tests against forest insect pests with the new Bt strain and improved, or experimental formulations started in 1971. Considerable more field testing will be done in 1972 against more forest pests.

The data from the 1971 tests indicate clearly that the new Bt preparation can readily achieve one control purpose, i.e. foliage protection.

Preliminary data from aerial tests of Dipel and Thuricide in 1972 gave indications of good foliage protection but relatively unsatisfactory population reductions of the gypsy moth. More work must be done with dosage, timing, and rates of application before a full evaluation of Bt can be made.

Phytotoxicity

No phytotoxicity has been reported for the new formulations of Bt.

In some cases, earlier formulations of Bt showed some phytotoxicity, but this was ascribed to adjuvants rather than the active ingredient.

Translocation

There have been no reports of Bt translocation in plants. Since the active ingredient is particulate and insoluble in commonly used carriers, translocation would be impossible.

Persistence in soil, water and plants

There is a scarcity of hard data on the longevity of Bt in the environment. The studies that have been made indicate that the spore will persist in soil for several weeks depending upon the soil type, soil flora, and extrinsic factors such as pH, moisture, and radiation.

No data is available on the survival of the crystal. However since the crystal is proteinaceous, enzymatic degradation by soil flora action can be presumed.

Survival of Bt on leaves is minimal when no additives are included in sprays. (17). Biological activity is usually restricted to 3-5 days. However, new formulations designed to protect Bt from the ravages of environmental factors have

shown considerable biological activity after 21 days of field exposure. Biological activity after one month is negligible.

Compatibility with other chemicals

Bt is incompatible with any chemical having a high pH (9.5 plus). The Bt products produced in the U.S. are compatible with most of the commonly used insecticides and fungicides. However the manufacturer of Bactospeine does not recommend that the material be mixed with other pesticides.

IV. Environmental Impact

A. In view of the body of safety data regarding the effects of Bt on humans, domestic animals, fish, birds, and beneficial insects which indicated that no ill effects were suffered by test animals, no monitoring of Bt used in forest insect control has been reported.

B. Inasmuch as Bt is exempted from tolerance, no residue analysis on food or feed has been performed when Bt has been used for forest insect control.

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APPENDIX 4

STUDY PLAN

FIELD EXPERIMENT OF INSECTICIDES ON PINE BUTTERFLY POPULATIONS BITTERROOT NATIONAL FOREST, MONTANA 1973

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Montana Division of Forestry

Date

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Date

STUDY PLAN
FIELD EXPERIMENT OF INSECTICIDES ON PINE BUTTERFLY POPULATIONS
BITTERROOT NATIONAL FOREST, MONTANA
1973

I. INTRODUCTION

Pine butterfly, Neophasia menapia (F. &F.), is one of the most damaging native defoliators of ponderosa pine, Pinus ponderosae Laws, in the western United States. This insect periodically reaches epidemic levels and has been known to cause extensive tree mortality (Hopkins 1907, Evenden 1940). Pine butterfly has been at an epidemic status on National Forest, State, and private lands in the Bitterroot Valley, causing severe defoliation for the past 2-3 years. In 1972, 40,000 acres of aerially visible defoliation occurred in Ravalli County (Bousfield and Dewey 1972). Some trees are entirely defoliated, appearing almost dead. Outlook for the summer of 1973 is that the infestation will continue--and possibly intensify. Tree mortality can be expected to increase each succeeding year the infestation persists.

Pine butterfly is not only a pest on ponderosa pine forests, it is also a pest of pines serving as shade trees in Missoula and the Bitterroot Valley. These trees enhance the value of the largely residential properties and their loss would require the cost of removal, as well as decrease the value of the properties. The costs of commercial tree removal is frequently several hundred dollars. Naturally, private landowners are deeply concerned about protecting individual trees on their properties.

In the 1950's DDT was effectively used to control epidemic pine butterfly populations (Cole, 1956). This material can no longer be used because of adverse environmental impacts. Currently no insecticides or biological materials are registered with the Environmental Protection Agency for

control of pine butterfly. As a result spraying to protect foliage is not now a management alternative. These field tests are designed to lead to the registration of one or more non-persistent materials for pine butterfly control to be available to forest managers and private homeowners if the need is warranted in future years.

II. PURPOSE

The purpose of the tests described in this study plan is to evaluate promising non-persistent insecticides and a new strain of Bacillus thuringiensis as suppression materials on epidemic pine butterfly populations, and to determine their effects on non-target insects, particularly the parasite-predator complex of the pine butterfly system.

Two methods of application will be used: (1) Aerial application using a conventional helicopter recirculatory spray systems, and (2) Individual tree application using conventional ground spray application equipment.

III. METHODS AND TREATMENTS

A. Aerial Applications

We will obtain field efficacy data for Zectran, and a commercial formulation of Bacillus thuringiensis aerially applied to pine butterfly infested ponderosa pine forests:

1. Zectran
   a. Fifteen hundreth's of a pound of Zectran dissolved into 1:9 solution of Dowanol TPM: deoderized kerosene to produce one gallon/acre (.15 lb/1 gal/acre).
   b. Three tenths of a pound of Zectran dissolved into 1:4 solution of Dowanol TPM: deoderized kerosene to produce one gallon per acre (.30 lb/1 gal/acre).

2. Bacillus thuringiensis

One pound of Dipel[1/] (Bacillus thuringiensis, HD-1 strain) dissolved into three gallons of carrier per acre (1.0 lb/ 3 gal/acre). The carrier will be a mixture of water (75%) and molasses (25%).

A conventional helicopter recirculatory spray system calibrated to deliver the above application rates will be used in this field test.

Zectran is commercially available, has undergone laboratory tests on the pine butterfly and extensive safety tests and field tests on other forest insects and could be registered for pine butterfly control in the minimum period of time.

Dipel is one of the commercial names for the new HD-1 strain of Bacillus thuringiensis. The isolation of the HD-1 strain along with the development of better wettable powders and emulsifiable concentrates have made present commercial formulations of B. thuringiensis 15 to 30 times more potent than those formerly used. In addition, the adoption of the International Unit of Potency (I.U.) to measure the relative effectiveness of different B. thuringiensis preparations eases the comparison of available commercial formulations against a target insect. Laboratory efficacy data and the potency of these present formulations expressed in I.U. must be obtained before they can be recommended for field tests against pine butterfly populations.

Laboratory tests of Dipel on pine butterfly larvae will be conducted in April by Dr. Sidney R. Siemer of Abbott's Laboratories. If it appears that the material can't be ready for field testing this year then one of the pyrethrin materials will be substituted for Dipel.

1/ Dipel is produced by Abbott Labs., North Chicago Illinois. Mention of a commercial product does not imply endorsement or approval of the product by the U.S.D.A. to the exclusion of others which may also be suitable.

B. Ground applications

Two dosages of four materials will be applied to butterfly infested ponderosa pine shade trees using conventional ground application equipment. The selection of dosages is based partly on relative toxicity of the insecticides to pine butterfly and partly on costs of application. The following table presents the four materials, the recommended dosages and their comparative costs.

Table 1. A list of insecticides and dosages selected for the ground application test and their respective costs.

Material	Cost/lb. Technical	Dosage	Cost of insecticide per gal. of final mix	Cost of insecticide at 10 gal. spray per tree.
Stabilized Pyrethrins (Formula 7083)	$50.00	.01 lb/10 gal	.050	.50
		.02 lb/10 gal	.10	1.00
Resmethrin (24.3% SBP-1382-2EC)	48.00	.01 lb/10 gal.	.048	.48
		.02 lb/10 gal.	.096	.96
Zectran 2E 2 lb/gal.	7.50	1 qt/100 gal. (.05 lb/10 gal.)	.037	.37
		1½ qt./100 gal. (.075 lb/10 gal.)	.056	.56
Malathion	1.00	1% (.83 lb/10 gal)	.083	.83
		¼% (.21 lb/10 gal)	.021	.21

The specific pyrethrin and resmethrin formulations that will be used in the tests are: (1) for pyrethrins, MGK Pyrocide F-7083 in a stabilized 1.4% emulsifiable concentrate; and (2) for resmethrin, 24.3% SBP 1382-2EC. These concentrates will be diluted by water to obtain the desired dosages.

Resmethrin and stabilized pyrethrin have high potential for pine butterfly control based on toxicity tests in the laboratory (Lyon and

Brown, 1971). These chemicals have undergone and passed field safety tests but have not been tested in the field against pine butterfly populations. Registration for use against ornamentals is underway. The extensive testing of Zectran has already been mentioned and there is substantial experience in the use of malathion on forest and shade tree pests.

C. Evaluation

The field effectiveness of these formulations will be evaluated by determining:

1. The relationship between pine butterfly larval mortalities and deposited dosage for the aerial treatments.
2. The differences in residual pine butterfly larval population densities in the treated and check areas.
3. The differences in defoliation damage caused by pine butterfly feeding between study trees in the treated and untreated areas.

The effects of the insecticide formulations on the parasite-predator complex of the pine butterfly will be determined by comparisons of:

1. The incidence of parasitism between pre- and post-spray populations of pine butterfly larvae for each treatment.
2. Number of known parasites and predators collected from drop trays placed in the treated areas.

D. Experimental Design for the Aerial Application

1. Each treatment will be applied to pine butterfly infested ponderosa pine forests on forty-acre blocks.
2. The four treatments (including check) will be replicated three times for a total of twelve 40-acre treatment blocks or areas.

3. Each forty-acre block will contain fifty sample trees divided into five clusters of ten trees each.

4. Pine butterfly populations on each tree will be sampled by cutting six foliated branches during each sampling period.

Consequently, for each sampling period there will be sixty branches from each cluster, three hundred branches from each 40-acre block and nine hundred branches for each treatment.

Treatments will be assigned to the blocks at random. We plan to apply all the treatments for a particular insecticide in one day. For example, .15 lb of Zectran dissolved in one gallon of TPM per acre will be aerially applied to three 40-acre blocks and .30 lb of Zectran dissolved in one gallon of TPM per acre will be applied to three 40-acre blocks--all in one morning. The commencement of spraying will be timed to one hundred percent egg hatch of the pine butterfly for Zectran and Dipel.

E. Experimental Design for the Ground Application (Shade trees test).

1. Each treatment will be applied to a total of 10 trees.

2. There will be nine treatments (2 dosages of pyrethrins, 2 dosages of malathion, 2 dosages of Zectran, 2 dosages of resmethrin, and check) with ten replications per treatment (one tree per replicate). Unsprayed trees will serve as buffers between all sprayed trees.

3. Pine butterfly populations on each tree will be sampled by cutting six foliated branches using an extendable pole pruner during each sampling period.

Therefore, the design consists of nine clusters of ten trees each for a total of 90 trees. The choice of treatment for a particular cluster of

ten trees will be random. A total of 540 branches will be cut during each sampling period.

IV. Study Procedure

A. Plot and Study tree selection

Twelve 40-acre blocks of ponderosa pine forest infested with high populations of pine butterfly larvae will be selected within the outbreak on the Bitterroot National Forest near Hamilton, Montana. The selection will be done in a manner to minimize contamination from treatment on nearby acres and will be based on the following criteria:

1. Presence of comparable butterfly populations based on egg counts (15 eggs/5-inch branch tip minimums).
2. Lack of proximity to streams, private lands, beehives, and other sensitive areas.
3. Maximum acceptable current level of defoliation--up to one-half of old foliage removed; 1972 growth undamaged.

Each forty-acre block will be mapped and each 40-acre map will be grided into 20 two-acre blocks and numbered from one to twenty. Five two-acre blocks will be selected randomly from those blocks encompassing the host type and will be the focal point for each ten tree-cluster.

The approximate location of each selected focal point will be made in the 40-acre blocks and the cluster will comprise the ten nearest trees suitable for sampling. These selected trees should not be over-topped by adjacent trees and should otherwise reflect an open-grown status. They should be amendable to sampling by a 24 foot-extendable pole pruner equipped with a cloth basket just below the cutting head. The location of these clusters and their study trees will be appropriately marked and

mapped. The sample trees should be numbered consecutively from 1-50 for each 40-acre block regardless of cluster.

Trees to be treated by ground applications in the shade tree test will be selected on the basis of accessibility for ground equipment.

B. Population Sampling

Pine butterfly larval populations will be sampled 48 hours prior to spraying and 3,7, and 14 days following spraying on the treated areas. The insecticidal activity of Zectran is usually completed within three days following spraying. However, it will take B. thuringiensis much longer to be effectively expressed in the treated pine butterfly populations. In order to compare the effects of these materials on the mortality rate of the pine butterfly populations over time it is necessary to sample all treated (including check) populations at the same periods following treatment. We recognize there may be some difficulty in obtaining a valid 14 day post spray sample due to tree condition and larval dispersal which occurs during the later instars. As a consequence we may have to adjust the sampling schedule described here.

Six branch samples, each approximately 15 inches long, will be cut from the crown of each sample tree using an extendable pole pruner. Each branch should drop into the cloth basket after cutting and then be carefully lowered to the ground. Any branch falling out of the basket should be discarded and another branch cut in its place. The length of the foliated portion of the branch sample will be measured to the nearest one-half inch. The contents of the cloth basket including the branch sample will then be placed into a polyethylene bag, accompanied by a card with the following information:

1. Date

2. Spray Block (40 acres) number

3. Cluster number

4. Tree number

5. Branch number (1-6)

6. Branch measurement (length of foliated portion).

The sample containing bag will be secured to prevent the escape of any larvae and placed into a burlap feed sack containing other branch samples from that study tree and cluster. Usually 15-20 bags can be placed in a sack depending on the size of the branches. The sacks containing the branch samples should be placed in a cool shady situation until all the population sampling on the 40-acre tract is completed. The samples are then taken to the field laboratory for processing before population sampling begins on the next 40-acre tract.

C. Processing Field Samples

1. The samples should be sorted according to tree. Tree numbers are then assigned to the insect counters so that each individual will examine the foliage from the same tree for the pre-spray and post-spray larval counts.

2. The sample branches are removed from the polyethylene bags. All insects are removed from the branches and bags and placed into petri-dishes by foliage examiners under the supervision of an entomologist. Each sample branch will be rated according to the defoliation classification scheme developed by Bousfield and Dewey (1972). See assessment of Foliage Retention section for further details. It is important to have enough foliage

examiners (insect counters) so that the samples can be rapidly processed. This will keep the need for sample storage at a minimum and reduce the probability of significant larval mortality or escape occuring between the time the samples are taken in the field and the examination of them in the laboratory.

3. The identification card accompanying the branch sample is attached to the petri dish (dishes) containing the insects removed from the branch samples and given to the supervising entomologist. The entomologists separates the pine butterfly larvae from other insects found in the samples. He counts the pine butterfly larvae and identifies and counts the insects found associated with the pine butterfly larvae and records this information on the identification card.

4. The entomologist also enters sample branch measurements, pine butterfly and associated insects counts on appropriate data form. He then places the pine butterfly larvae into rearing to determine the incidence of parasitism in the sampled population.

The samples not processed by the end of the working day are placed over-night in a walk-in cooler. The temperature of the cooler should be between 40° and 45° Fahrenheit.

D. Sampling and Assessing Deposited Dosage

The best systems available to assess B. thuringiensis deposit or coverage will be used after consultation with Dr. Hank Thompson of the PNW Forest Insect Disease Project and with DTC Ft. Douglas, Utah. The methodology followed by PNW-2208 (AAP) for the 1972 field tests of Zectran on the Douglas-fir tussock moth will be described here for Zectran. Foliage

samples, aluminum plates, and white kromekote cards will be used for the quantitative spray deposit assessment. The foliage samples will be collected immediately after spraying from the same 50 trees per 40-acre tract used for the larval and pupal samples. The samples will be taken from the four cardinal directions of each tree.

The laboratory procedure described by John Neisess of PNW-2208 required to estimate the amount of deposited insecticide from the foliage and aluminum plates will be followed. The amount of Zectran from the foliage samples will be estimated as ug of material/gram of foliage. The amount deposited on the aluminum plates can be expressed in terms of gallons per acre (gpa) since the exact area of the sampling surface will be known. The number of visible drops per square cm. on the kromekote cards can be counted under a dissecting microscope.

E. Assessing Effects on Non-target insects

A sub-sample of the pine butterfly larvae collected from the branch samples will be placed into rearing for parasite emergence. The incidence of parasitism will simply be a relation between the number of insects placed in rearing and the species and number of parasites that emerge. Collections of insects from drop trays placed beneath selected study trees will be examined for parasites and predators associated with the pine butterfly.

F. Assessment of Foliage Retention

1. Trees and Branches

Defoliation estimates or assessments of foliage retention will be done in several ways.

a. Whole tree visual classification of defoliation and damage according to the following categories developed by Bousfield and Dewey (1972):

0 - Green, no visible defoliation (negligible)

1 - Less than one-half old foliage removed; 1973 growth intact (light).

2 - More than one-half old foliage removed; 1973 growth intact (moderate).

3 - Most of old foliage removed; less than one-half new foliage removed (heavy).

4 - Most of old foliage removed; more than one-half new foliage removed (severe).

Plot and block defoliation indexes are the mean of the individual tree ratings. Tree ratings will be made in late July when pupation is well along and defoliation is completed.

b. The above defoliation classification will be used to assess the change in foliage retention during the pre- and post-spray sampling periods for each tree. Each foliated branch collected during each sampling period will be examined and placed into one of the above five categories. The counts or number in these categories will be tested by statistical analyses to see if they differ signficantly between sampling periods for each treatment, and between treatments for the post-spray-sampling period.

2. Aerial photography

False color aerial photography has been shown to be a valuable tool for assessing effects of aerial sprays applied to suppress forest defoliators by providing estimates of foliage saved in spray blocks as compared with surrounding areas which have not been treated.

Aerial photography of each 40-acre spray block will be obtained with Kodak Ektachrome infrared Aero film, type 2443, at a scale of 1:15,840 during late July when the pine butterfly larvae have completed their feeding and defoliation is most conspicuous. Aerial photos will be examined in stereo to determine area (acres) protected by the spray application within the designated spray block and adjacent pine stands. Maps showing areas of foliage protection, degrees of foliation and zones of spray drift in relation to the spray blocks will be prepared in accordance with procedures described by Ciesla et al. (1971).

V. Data Analyses

Various kinds of data will be produced by the sampling efforts in this field experiment. Some of them are:

A. Estimates of pre- and post-spray larval population densities for each tree, cluster, and 40-acre treatment block. The estimates of population density will be made from population counts expressed as number of pine butterfly larvae-pupae per 100 inches of foliated branch length.

B. Estimation of mortality (population response to the insecticide) by ratio estimation, e.g. the survival rate is given by

$$r_i = (X_{2i}/Y_{2i})/(X_{1i}/Y_{1i})$$

Where X_{1i} and X_{2i} denote pre- and post-spray insect counts and where Y_{1i} and Y_{2i} denote pre- and post-spray measurements of foliated branch length for the ith tree cluster at each treatment area. Survival ratios will be used rather than percentage reduction per se, so as to provide a comparison on the same basis as actual survival counts.

Aerial mapping ... will be

obtained with ... at a scale of 1:15,840 during late July when ...

larvae have completed their feeding ... conspicuous. Aerial photo ...

determine area (acres) protect...

the designated spray block ... showing areas of foliage protection ... zones of spray drift ... prepared in accordance with ... (1971).

Data Analysis

... kinds of data will be produced ... pertinent. Some of them are:

A. Estimates of pre- and post-spray larval ... tree, cluster, and 40-acre treatment block. ... will be made from population counts expressed ... larvae-pupae per 100 inches of foliated ...

B. Estimation of mortality (population response ... insecticide) ... estimation, e.g. the survival rate is given as

$$= [(y_{2i}/x_{2i})/(y_{1i}/x_{1i})]$$

and x_2 denote pre- and post-spray insect counts and where y_{1i} ... pre- and post-spray measurements of foliated branch length ... tree cluster at each treatment area. Survival ratios will be ... per se, so as to permit comparison

C. Estimates describing a range of deposited dosage for each sample tree and tree cluster for the aerial application treatments. The relationships between these sets of data will be described by regression techniques.

D. Visual estimates classifying tree defoliation caused by pine butterfly feeding.

1. Aerial photographs

2. Classification by undamaged, partly damaged and severely damaged categories tested by co-variance analyses and the Duncan Multiple Range Test.

E. Incidence of parasitism expressed as number of parasites (by species) per 100 pine butterfly larvae and pupae between pre- and post-spray populations of pine butterfly larvae for each treatment.

Most comparisons of population parameters and foliage depletion among the treated areas can be made by analyses of variance and co-variance.

VI. Cooperation

This field experiment will be a cooperative venture between the Insecticide Evaluation Project(PSW-RWU-2203), Abbott's Labs., Division of State and Private Forestry Region One, Montana Division of Forestry, Forest Insect Disease Project (PNW-2203), Missoula Equipment and Development Center (MEDC) and U. S. Army Deseret Test Center (DTC). The contractual arrangement for aircraft, calibration, and determination of spray atomization, swath width and deposit patterns for this equipment will be the responsibility of Forest Pest Control specialists of Region One, and MEDC.

Region One will provide the field crews needed to do the population sampling and damage assessments. The aerial photography work will be the responsibility of Region One. Region One will arrange for the field

laboratory and adequate transportation for the work activity. Training of field and laboratory crews will be the joint responsibility fo Region One and Insecticide Evaluation Project (IEP).

Region One will locate the study areas and trees. IEP in consultation with Pest Control specialists of Region One will devise the experimental design and will provide the data forms, and do the analyses for estimating treatment effects. Region One and DTC, will assess the deposited dosage and IEP will do the appropriate analyses of all the data. However, the data should be freely available to both Region One and IEP. Publication of the results of this field test will be under the joint authorship of the appropriate IEP and Region One personnel and others.

Publicity and public involvement will be the responsibility of Region One.

With the above discussion in mind the specific duties are as follows:

1. Design, planning and review of test. IEP (PSW 2203), R-1 Division of S&PF Bitterroot NF, INT., Montana Div. Forestry.
2. Development and conduct of inform and involve program. Bitterroot National Forest.
3. Preparation of appropriate environmental analyses. R-1 Division of S&PF, Bitterroot NF.
4. Contracting of aircraft. R-1 Division of S&PF
5. Conduct of ground spray test. Montana Division of Forestry.
6. Employment of temporary personnel, and Selection of field lab site. Bitterroot NF
7. Selection of spray blocks and heliport location. Bitterroot NF, R-1 Division of S&PF, IEP, Montana Div. Forestry

8. Calibration, determination of spray atomization, swath width, etc. MEDC, R-1, Division of S&PF.

9. Spray deposit assessment. U. S. Army Deseret Test Center, Fort Douglas, Utah; USFS: IEP

10. Meteorological support. U. S. Army Deseret Test Center, Fort Douglas, Utah, or U. S. Weather Service.

11. Aerial photography and photo interpretation. R-1 Division of Engineering and Division of S&PF.

12. Training and supervision of field crews. IEP, R-1 Division of S&PF.

13. Analysis of data and reporting of results. IEP, R-1 Division of S&PF, Montana Division of Forestry.

X. Tentative Financial Plan - Pine Butterfly Test

	F Y 1973	
Salaries	$25,392.	(16 field, 30 lab @2.30/hr for 30 days)
Helicopter time	4,200.	(840 acres @ 5.00/acre)
Chase plane	200.	
Chemicals	300.	
Vehicle rental	6,000.	(12 vehicles)
Carrier	320.	(1,600 gallons Deobase)
Lab and Cooler rental	400.	
Per Diem	3,500.	(@$20.00/day)
Equipment & supplies	2,500.	(Collecting gear, media, petri-dishes etc)
Computer time	600.	
Tree sprayer	500.	
Inform and involve activities	1,000.	
MEDC	1,300.	
Spray deposit assess	1,500.	
	$47,712.	

*Salaries of R-1 and IEP contributed

	F Y 1974
Aerial photo evaluation	500.
Computer time	600.
Preparation of report	1,300.
	$2,400.
Grand total	$50,112

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